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EFFECT OF SUBANESTHETIC NITROUS OXIDE—OXYGEN MIXTURES ON PAIN SENSITIVITY OF THE TOOTH[1]

ALFRED H. CHAMBERS AND GEORGE G. SCHULTZ

From the Department of Physiology and the School of Dentistry, University of Pennsylvania, Philadelphia, Pa.

The effect of nitrous oxide on the pain sensitivity of the tooth is important. In testing the analgesic effects of nitrous oxide by pain sensitivity of the skin, there are many complicating factors. Use of the tooth, under appropriate stimuli, may offer the advantage of eliciting uncomplicated pain for such studies. For these reasons, the following experiments were undertaken.

METHODS

Nitrous oxide-oxygen mixtures, ranging in concentration from 10% to 70% nitrous oxide, were made in 2 ninety-liter spirometers. The subject inhaled the gas through a set of rubber flap valves fitted to a Heidbrink face mask and exhaled to the air. Leaks were avoided by the use of carefully constructed valves and a snugly-fitted mask.

The test stimulus was a high-frequency induction shock delivered by a standard clinical apparatus for determination of pulp sensitivity, the "Vitalometer." The ordinary contact point was modified by attaching to it a flexible extension wire tipped with a specially constructed contact that could be passed through the facepiece assembly and fastened securely to the tooth.

The routine of an experiment was as follows: A maxillary first incisor of a reclining subject was isolated with rubber dam, dried, and the contact point applied and secured with dental floss. Good contact with the tooth was assured by the use of Cambridge electrode jelly on the point. The same tooth was used in all the experiments on the same subject. After the mask and respiratory valves were applied, the spirometer valves were adjusted so that the subject inhaled room air. The subject gave a signal upon the recognition of 2 levels of sensation. The first was a just perceptible sensation without noticeable pain and the second, with increased stimulus strength, just bearable pain. The strengths of stimuli required to give these sensations were quite reproducible in any given experiment, although the second had a somewhat less sharp end-point than the first, and both could be maintained constant over periods of more than 30 minutes. After obtaining 3 or more constant values for both degrees of sensation over a period of 4 to 6 minutes, the subject inhaled the gas mixture. Sensation measurements were continued at 1 or 2 minute intervals for the duration of the gas inhalation, usually lasting from 6 to 15 minutes, and afterwards when the subject breathed room air again until the readings returned to control level, usually a matter of 5 to 10 minutes.

[1] Received for publication October 24, 1944.

RESULTS

Fig. 1. illustrates the effect of 50% nitrous oxide on the 2 levels of sensation during a typical experiment. The effects obtained with different gas mixtures and different subjects varied only quantitatively, the general course of all the experiments being quite similar, namely, a rapid increase in stimulus strength required for just bearable pain, a very strikingly smaller change for just perceptible sensation, and a rapid return of both levels to control values following inhalation of the gas mixture.

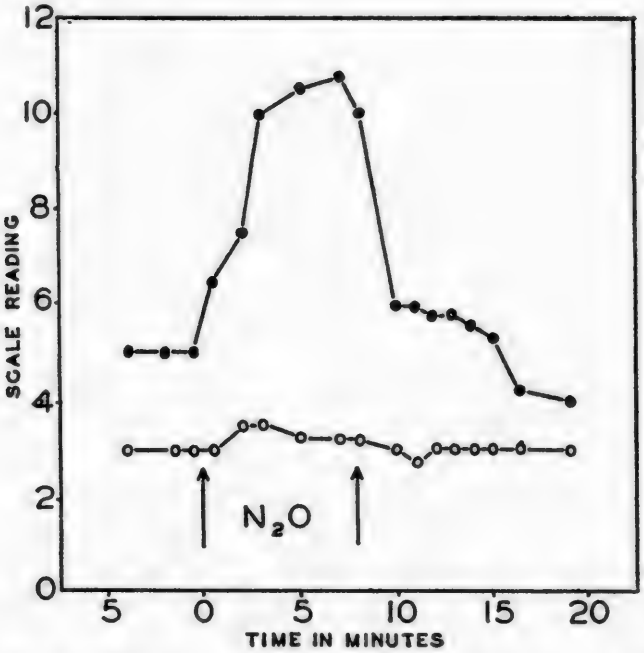

Fig. 1. Effect of 50% nitrous oxide on pain sensation. Solid circles = just bearable pain. Open circles = just perceptible sensation.

Results of all experiments on one subject are summarized in *fig. 2.* Each pair of points, except that at 15%, represents the average of 2 or more trials. Total ranges for just bearable pain were quite large, although values during a given experiment were quite constant. The ranges for just perceptible sensation were considerably smaller. The large day-to-day variations, coupled with the relatively small number of trials with each concentration, undoubtedly account for the apparent anomaly of less effect with 40% than with 30% nitrous oxide.

Along with the changes in sensation signaled by the subject, there were a number of subjective sensations which cannot be described in detail. Inhalation of nitrous oxide in concentrations greater than 20% is accompanied by a peculiar throbbing and tingling sensation that seems to reach a climax and then subside somewhat. Seevers, Bennet, Pohle and Reinardy (1) noticed a similar effect.

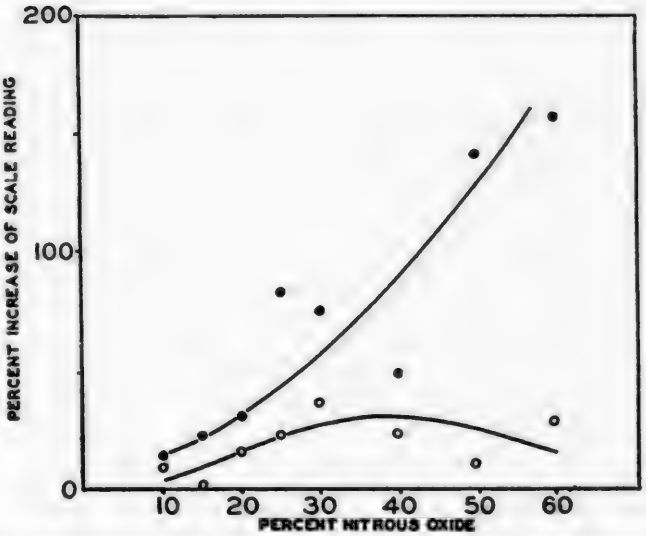

Fig. 2. Change in threshold for just bearable (solid circles) and just perceptible sensation (open circles) with varying concentrations of nitrous oxide.

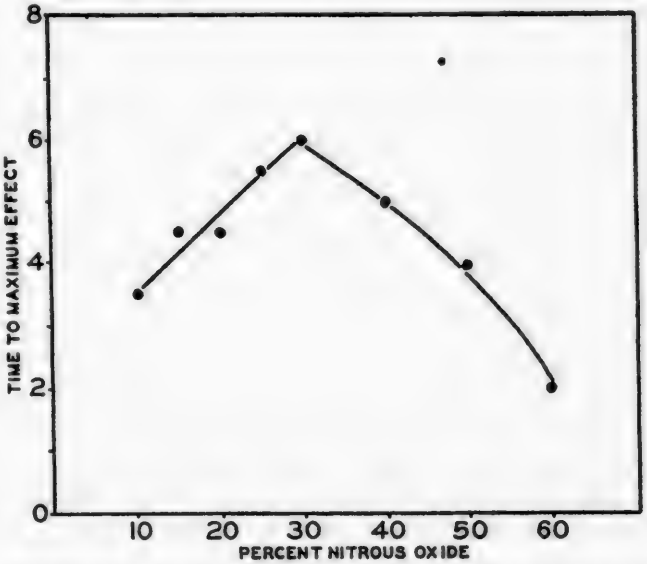

Fig. 3. Time (minutes) required to reach maximum increase in threshold for just bearable pain.

The stimulus strength for just bearable pain showed a similar rise to a peak followed by a slight fall in many experiments, although it cannot be said that these 2 findings coincided.

When 60% and 70% nitrous oxide were inhaled, amnesia occurred as evidenced by the subject's failure to remember statements made after about a minute following the start of the gas inhalation. In addition, there was no memory of any stimulation of the tooth, painful or otherwise, and the signals given by the subject during stimulation were confused at times.

Inhalation of the gas in concentrations up to 60% never caused any objectionable reactions or sensations in either of the 2 subjects used. Seventy per cent inhaled by one subject for 6 minutes resulted in considerable dizziness and some nausea lasting about 90 minutes after cessation of the inhalation.

Fig. 3. illustrates the time required to reach maximum analgesic effect following the start of inhalation. The effect measured does not represent that produced when the tissues are in equilibrium with the particular gas mixture used, attainment of which would presumably occur only after a much longer time. For practical purposes, however, the effect during the first 15 minutes is of greater importance and probably is very nearly the same as that at equilibrium.

DISCUSSION

One of the most interesting, and unexpected, findings in this study is the great difference in effect of the nitrous oxide on the 2 degrees of sensation measured. In view of the evidence of Pfaffman (2) and others that the cat's tooth possesses only pain receptors, receptors for other sensations being present in the periodontal structures, it is quite likely that only one type of receptor is being affected by our method of stimulation, the 2 levels measured being different degrees of the same sensation rather than different sensations. The mechanism causing this difference in effect on the 2 levels of sensation most probably lies in the central nervous system. Forbes and Miller (3) have shown that ether anesthesia does not significantly affect the nerve impulse in the peripheral axon, but does prevent the arrival of impulses at the pons. Observations of Derbyshire, Rempel, Forbes and Lambert (4) on the effect of ether, pentobarbital, and avertin on cortical potentials indicate that these anesthetics block afferent impulses before their arrival at the cortex or at some point in the cortex. Since it is unlikely that nitrous oxide has a qualitatively different action from other anesthetic drugs, our results can be explained by the failure of the synapses to transmit afferent impulses of high frequency but not of low frequency when affected by the nitrous oxide, or on the basis that the cortical interpretation as just bearable pain of impulses originating in the tooth pulp is greatly subject to influence, whereas, the mere registration of impulses as perceptible sensation is influenced to a much lesser degree.

The results illustrated in *fig. 3* are of considerable interest, but possible explanation of them is withheld until more data are available for their interpretation.

Application of these results to clinical practice presents considerable difficulty. It is virtually impossible to make any general statement regarding the degree of analgesia produced by any given concentration of nitrous oxide owing to considerable individual variation, day-to-day variation in an individual, and variation in degree of painful stimulation produced by different surgical procedures.

As far as pain sensation in the tooth is concerned, it can be stated that concentrations of nitrous oxide up to 30% would very likely have little practical analgesic value for extraction or deep cavity preparation. On the other hand, stimuli strengths required to elicit just bearable pain when inhaling 50% and 60% nitrous oxide are such that, if required in an individual not under the influence of any analgesic agent, serious impairment of the pulp sensitivity would be indicated. A somewhat more certain estimation of the analgesic value of the gas was afforded by one of our subjects who underwent extraction of a mandibular third molar while inhaling 60% nitrous oxide and reported complete absence of any sensation of pain during the operation.

In view of this observation there arises the definite possibility of performing many dental procedures under concentrations of nitrous oxide which preclude unnecessary exposure of the patient to the very real danger of the severe anoxia which usually accompanies present day administrations and which constitutes the only serious hazard in the administration of this gas to normal individuals.

SUMMARY

1. The effect of subanesthetic nitrous oxide-oxygen mixtures on the pain sensitivity of the tooth was determined.

2. Sensation resulting from weak stimulation was found to be affected less by a given gas mixture than that from strong stimulation.

3. Analgesia sufficient for extraction was found to be produced by 60% nitrous oxide, and a significant degree of analgesia by mixtures containing not less than 30% to 40% nitrous oxide.

4. Maximum effect on pain sensitivity was reached within 2 to 6 minutes, depending on the gas mixture, and returned to normal within 5 to 10 minutes.

5. The application of these findings to clinical practice is discussed.

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CLINICAL OBSERVATIONS ON PIGMENT VARIATIONS IN HEALTHY ORAL TISSUES OF THE NEGRO

CLIFTON O. DUMMETT, SR., B.S., D.D.S., M.S. D.

Department of Oral Health, Meharry Medical College, Nashville, Tenn.[1]

Complete and detailed information is lacking on the normal appearance and color of the various tissues in the oral cavity of various ethnic groups. This is notable especially in references to the color of the gingivae and gums[2] where the pink oral mucosa of the Caucasian is considered the desired norm for judging healthy oral tissues.

Among 11 texts on oral pathology, diagnosis and periodontia, only 3 mention variations in pigmentation of the oral mucosa. The variations that occur in the color of the gums and gingivae of so many members of some white and most non-white groups make it imperative that more consideration be given to the normal appearances of these and the other oral tissues of these people. Since knowledge of the normal is an essential prerequisite to diagnosis of the abnormal, it is suggested that more attention and study be devoted to the appearance of healthy pigmented tissues so that some basis may be available for diagnosing the clinical signs of incipient disease when the latter occurs in the mouths of these particular individuals.

The following is a preliminary study[3] of the various pigment variations in the gingivae, gums and buccal mucous membranes of 50 Negro patients presenting at the dental clinic of Meharry Medical College. The patients were carefully selected in order to obtain tissues clinically as healthy as possible. In every case, the oral examination was made on the labial and lingual tissues of the 6 anterior teeth from the distal of the right canine to the distal of the left canine in both upper and lower arches. The occlusion of these teeth was normal. No calculus was present on these anterior teeth in any of the cases and all the patients gave histories and clinical evidences of excellent oral hygiene. Clinical normality was judged by the absence of any tissue hypertrophy, atrophy, gingival recession, or tooth mobility. The interproximal spaces were well-filled with healthy tissue up to the contact points, and instrumental examination of the gingival crevices produced no hemorrhage. Cases which did not meet all of these qualifications were not used in this study.

In Table I, the sex, occupation, age, color of gingivae, gums and buccal mucous membrane, density and superficial appearance of the gingivae and gums, depth of the gingival crevice, use of drugs, and presence of systemic disease have been recorded. The color of the tissues was examined under intra-

[1] Received for publication September 13, 1944.

[2] For the purposes of this paper gingivae, the tissues approximately 2 mm. in width surrounding the cervices of the teeth, and the gums, the tissues extending apically from the gingiva and finally joining the oral mucous membrane.

[3] (There are in progress other studies on the pigment variations in the remaining oral tissues and on the relationship between these pigmentations and skin color.)

oral illuminators; while the density and superficial appearance were obtained by palpation and instrumental and visual examination. The depths of the gingival crevices were ascertained by periodontal membrane explorers. Because of the tendency of certain drugs to produce oral tissue discolorations, it was thought advisable to record the patient's history of the use of drugs. Finally, there is a record of the presence or absence of systemic disease. The reasons for including such data in this study are obvious since the color changes that appear in these tissues as a result of such pathological conditions as Addison's disease, the blood dyscrasias, and certain of the vitamin deficiencies would confuse the issue.

OBSERVATIONS AND DISCUSSION

Examination of Table I shows that the color of the gingivae varied from 'very light pale pink' to 'purplish black'. Approximately 66% of the cases examined possessed gingivae with different degress of a pink coloration devoid of melanogenous pigmentation. The color of the gums, also, varied from 'very light pale pink' (Case 7) to 'purplish black' (Case 13). Approximately 72% of the cases examined showed gums with some degree of melanogenous pigmentation. There were innumerable variations of this pigmentation, and there were some cases where definite areas of pigmentation and just as definite areas of non-pigmentation were found in the same mouth. This was often evidenced as pigmentations of a few gingival crests or interproximal papillae, or isolated spots in the gums, and non-pigmentations of other similar regions (Cases 30, 34, 35). The color of the mucous membranes varied from 'pale pink' (Case 7) to 'purplish pigmented grayish pink' (Case 32). About 18% of the cases examined showed mucous membranes with some degree of pigmentation. Although there were one or two cases where light pink mucous membranes were seen in mouths in which the gingivae and gums were heavily pigmented (Case 39), it seemed that light pink mucous membranes were more often found in cases where both gingival and gum pigmentations were either slight or entirely absent.

With regard to the amount of pigmentation in various parts of the oral cavity, it appeared that the canine region in both upper and lower arches was the most heavily pigmented region in the mouth. In the majority of cases studied, the amount of pigmentation was less on the lingual than on the labial, even though the general pattern was often similar. In addition to this, the pigmentation in the posterior gum regions was somewhat lighter than in the anterior. Finally, pigmentation was seen just as consistently in both partially edentulous areas and completely edentulous mouths as it was seen in the gingivae and gums of individuals with complete dentitions. This is rather interesting when compared with the relative lack of pigmentation in edentulous areas in cases of mild bismuth stomatitis. In bismuth stomatitis, the gingivae are characteristically pigmented around existing teeth.

This limited study stimulates further questions on racial pigmentation. One might question the reasons for the canine area being the region of greatest pigment deposition. One may also wonder whether the lesser amount of exposure of the lingual and posterior regions of the mouth to the effects of light,

TABLE I

Data on pigmentation of gingivae, gums and oral mucosa

CASE	SEX	OCCUPATION	AGE	GINGIVAE	GUMS	BUCCAL MUCOUS MEMBRANE	DENSITY OF GINGIVAE AND GUMS	USE OF DRUGS	CREVICE DEPTH AT DEEPEST POINT	SYSTEMIC DISEASE
									mm.	
1	F	College student	19	Uniform purplish black	Purplish black and dark pink areas	Pink	Firm and rough	Tobacco, tea, coffee	.8	None
2	F	Student nurse	19	Dark pink	Pigmented purplish brown	Pink	Trifle soft	None	.9	None
3	F	Housewife	32	Uniform pale pink	Pink with some brown pigmentation	Slightly grayish pink	Firm and rough	Anacin, coffee, tobacco	.9	Gastric tumor
4	F	Student nurse	20	Light pink	Dark purple pigmentation	Pink with slight purple pigmentation	Trifle soft and spongy. Rough	Aspirin, tea	1.0	None
5	F	Student nurse	20	Purplish black	Lighter purple	Pink with slight purplish pigmentation	Firm and rough	Aspirin, tea, coffee	.8	None
6	F	Dental clerk	21	Pink with slight brown pigmentation	Red	Pink	Firm and rough	Aspirin	2.0	None
7	F	Housewife	21	Very light pale pink	Very light pale pink	Pale pink	Firm and slightly rough	None	.9	None
8	M	Student	22	Uniform dark pink	Light pink	Light pink	Firm and rough	None	.9	None
9	M	Dental student	23	Pink with slight purple pigmentation	Pink with slight purple pigmentation	Light pink	Firm and rough	Coffee, tea, tobacco, alcohol occasionally	.9	None
10	M	Medical student	24	Light pink	Pink with purple spots	Light pink	Trifle soft and spongy. Rough	None	.5	None
11	M	Dental student	24	Light pink	Light pink with purplish black areas	Slight purplish gray	Firm and rough	Tobacco, alcohol	.9	None
12	M	Dental student	24	Pink	Pink	Pink	Trifle soft and spongy. Slightly smooth	Tobacco	.8	None

TABLE I—*Continued*

CASE	SEX	OCCUPATION	AGE	GINGIVAE	GUMS	BUCCAL MUCOUS MEMBRANE	DENSITY OF GINGIVAE AND GUMS	USE OF DRUGS	CREVICE DEPTH AT DEEPEST POINT	SYSTEMIC DISEASE
									mm.	
13	M	Dental student	25	Purplish black	Purplish black with darker areas	Slightly purplish gray pigmentation	Firm and rough	Tobacco, coffee, tea	.8	None
14	F	Dental clerk	31	Light pink	Slightly darker and stippled pink	Light pink	Firm and rough	Coffee, tea, aspirin	.9	None
15	M	Dental student	31	Dark purple	Dark pink	Slight purple pigmentation	Firm and rough	Tobacco, tea, alcohol	.8	None
16	F	School teacher	32	Light pink	Light pink	Light pink	Firm and rough	Coffee, tea	.8	None
17	F	Student	17	Red	Red with brown pigmentation	Slightly purplish pigment	Firm and rough	Coffee	1.0	None
18	F	Housewife	37	Pink	Pink with very slight brown pigmentation	Pink	Firm and rough	Coffee, tea	.9	Indigestion
19	M	Dental student	24	Light reddish pink	Brownish pink	Pink	Firm and rough	Tea, tobacco	.9	None
20	F	Student	22	Brownish pink	Reddish pink	Grayish pink	Slightly soft and rough	Tea, tobacco, alcohol	.9	None
21	F	Clerk	24	Light pink	Reddish pink with occasional purplish pigmented spots	Pink	Firm and rough	Tea	.9	None
22	F	Teacher	21	Brownish pink	Reddish pink	Pink	Firm and rough	Tea, aspirin	.9	None
23	F	Housewife	26	Light pink	Brownish pink	Pink	Firm and rough	Coffee, tea, tobacco	.9	None
24	F	Student	19	Light pink	Pink with purplish spots	Grayish pink	Firm and rough	Coffee, tea, tobacco	1.0	None
25	F	Student nurse	21	Pink	Uniform purplish black	Reddish pink	Slightly soft and spongy. Rough in appearance.	None	1.0	None
26	F	School hurse	25	Light pink	Pink with large purplish black areas	Grayish pink	Firm and rough	None	.9	None

TABLE I—*Continued*

CASE	SEX	OCCUPATION	AGE	GINGIVAE	GUMS	BUCCAL MUCOUS MEMBRANE	DENSITY OF GINGIVAE AND GUMS	USE OF DRUGS	CREVICE DEPTH AT DEEPEST POINT	SYSTEMIC DISEASE
									mm.	
27	M	Student	22	Light pink	Light brownish pink	Reddish pink	Firm and rough	Tobacco, alcohol occasionally	.85	None
28	M	Teacher	25	Light pink	Purplish brown pigmentation	Reddish pink	Firm and rough	Coffee, tea	.8	None
29	F	Student	21	Reddish pink	Reddish with slight brown pigmentation	Reddish pink	Firm and rough	Tobacco, alcohol occasionally	.9	None
30	F	Teacher	28	Light pink with purple pigmentation of a few papillae	Light pink with definite purplish black areas	Grayish pink	Firm and rough	Coffee, tea	.5	None
31	M	Student	20	Very light pink	Very light pink	Slightly grayish pink	Firm and rough	Tobacco, tea. Alcohol occasionally	.9	None
32	F	Student	17	Purplish black with a few pink areas	Purplish black with a few pink areas	Gray-pink. Slight purple pigmentation	Firm and rough	Tobacco, tea, coffee. Alcohol occasionally	.9	None
33	M	Student	24	Light pink	Reddish pink with slight brown pigmentation	Light pink	Firm and rough	Tobacco, coffee, tea	.5	None
34	M	Student	24	Purple with a few pink areas	Purplish black	Grayish pink	Firm and rough	Coffee, tea. Alcohol occasionally	.9	None
35	M	Student	21	Light purple with a few pink areas	Uniform purple pigmentation	Grayish pink	Firm and rough	Tobacco, coffee, tea. Alcohol occasionally	.9	None
36	F	Nurse	23	Reddish pink	Brownish pink	Light pink	Firm and rough	Aspirin, tea	.5	None
37	M	Student	24	Reddish pink	Gray-pink with purple pigmentation	Light pink	Firm and rough	Tobacco, tea. Alcohol occionally	.9	None
38	F	Nurse	28	Light pink	Reddish pink with a few brownish areas	Grayish pink	Firm and rough	None	.9	Migraine
39	F	Student	22	Brownish purple with a few pink areas	Brownish purple	Light pink	Firm and rough	None	.9	None

TABLE I—*Concluded*

CASE	SEX	OCCUPATION	AGE	GINGIVAE	GUMS	BUCCAL MUCOUS MEMBRANE	DENSITY OF GINGIVAE AND GUMS	USE OF DRUGS	CREVICE DEPTH AT DEEPEST POINT	SYSTEMIC DISEASE
									mm.	
40	F	Housewife	24	Light pale pink	Light pink with a few brownish spots	Light pink	Firm and rough	Coffee, tea, tobacco. Alcohol occasionally	.5	Spastic colitis
41	M	Student	25	Purplish pink with a few pink areas	Uniform purple	Pink	Firm and rough	Tobacco	.7	None
42	M	Student	20	Purplish red	Purplish red with brown pigmentation	Grayish pink	Firm and rough	Tobacco, tea occasionally	.8	None
43	F	Nurse	21	Light pink	Pink, slight brown pigmentation	Reddish pink	Firm and rough	Tobacco, coffee. Tea occasionally	.8	None
44	M	Student	16	Very light pale pink	Very light pale pink	Light pink	Firm and rough	Tea occasionally	.8	None
45	F	Clerk	27	Very dark reddish purple	Very dark reddish purple	Gray-pink, purple pigmentation	Firm and rough	Tobacco	.8	Constipation, hypertension
46	F	Nurse	22	Light reddish pink	Reddish pink	Pale pink	Firm and rough	Tobacco, coffee, tea, aspirin	.8	Constipation, dysmenorrhea
47	F	Housewife	23	Light pink	Light pink	Grayish pink	Firm and rough	Tea occasionally	.8	None
48	F	Clerk	32	Light pale pink	Pale pink with slight brown areas	Light grayish pink	Firm and rough	Coffee, tea, tobacco	.8	Constipation occasionally
49	F	Student	21	Light pink	Slight reddish pink	Reddish pink	Firm and rough	Tea occasionally	.8	Indigestion, spastic colitis
50	F	Nurse	19	Purplish pink	Deep purplish brown	Grayish pink	Firm and rough	Tobacco occasionally	1.0	None

variations in temperature, and the irritating influences of dust particles, etc., could be responsible for the lesser amount of pigmentation. Observations have been made on the relationship of gum pigmentation to skin color. In the cases seen in the present study, it would appear that although there is no consistency in the relationship, gum pigmentation does seem to be related to the color of the skin. Although a few very dark complexioned individuals possessed perfectly

pink gums devoid of any melanogenous pigmentation, more often the darker the individual the greater the possibility of pigmentation of the gingivae. Whether or not heredity plays the most important part in the transmission of gingival melanogenous pigmentation and leads to classification as a racial characteristic is a question that needs more thought and further investigation.

There is no logical basis for the beliefs that pigmented gingivae are always diseased, that individuals with pigmented gingivae require more anesthetic solution than those with non-pigmented gingivae, nor that bites received from individuals with oral mucosa pigmentation are per se fatal.

SUMMARY AND CONCLUSIONS

There is need to know more about the color of the oral tissues in some white and most non-white groups in which the normal color of these tissues is very often different from that mentioned in most textbooks. As a result of a short preliminary clinical study of the healthy tissues of 50 Negro patients, it was found that the color of the gums and gingivae in the healthy mouths of this particular group presented considerable variations that ranged from a very light pale pink to degrees of purplish-black pigmentation.

Gums and gingivae showing little or great pigmentation are perfectly normal, except in those cases where histories of local and systemic disease would intimate that these particular conditions are responsible for the production of this discoloration. Diagnosis of disease solely on the basis of color changes are, therefore, difficult and unreliable.

These facts should be advantageously presented in textbooks so as to discountenance popular opinions and superstitions that are, to say the least, illogical and unsound.

BACTERIOLOGICAL APPRAISAL OF ADHESIVE DENTURE POWDERS[1]

HENRY A. BARTELS, B.S., D.D.S.

Department of Oral Pathology, School of Dental and Oral Surgery, Columbia University, New York, N. Y.

Adhesive powders are employed by individuals to improve the stability of their dentures. When such powders are sprinkled on the mucosal surfaces of dentures and the latter inserted in the mouth, the adhesive powder absorbs moisture from the saliva adhering to the mucous membrane and swells to form a mucilaginous menstruum. What effect has the ingredients of such a menstruum on the microorganisms in contact with it? Such a question was propounded recently by Professor W. W. Hurst of the prosthetic department of Western Reserve University, in a discussion of an article by the writer.[2] Since no information was available on this subject, the following experiments were performed with 4 brands of adhesive powder bought on the open market (Wernet's powder, Co-re-ga, Holdent and Fasteeth).

Experiment 1: Loopfuls of a 24 hour old broth culture of *Staphylococcus aureus* were inoculated in parallel streaks on the surface of beef infusion agar. An adhesive powder was then distributed by means of a small bore capillary pipette in narrow rows perpendicular to the streaks. Thus, at various points on the medium, there were sites of intersection of the staphylococcus inoculum and the adhesive powder. The plates were incubated for 24 hours.

Experiment 2: The above experiment was repeated employing the gram negative, *E. coli*, (*B. coli*) as the inoculum. *Results:* The adhesive powder absorbed moisture from the medium and formed a mucilaginous menstruum similar to that found on dentures The gram positive, *Staphylococcus aureus*, as well as the gram negative, *E. coli*, grew vigorously. Growth was not affected where the streaks came in contact with the adhesive powder of any of the 4 brands tested.

Experiment 3: The technique of Experiment 1 was repeated employing blood agar medium and fresh saliva as the inoculum. A control plate was inoculated with an equal volume of saliva. *Results:* The microorganisms, which were present in the specimen of saliva and which grew aerobically on blood agar, showed no inhibition. The colonies of these microorganisms were numerous and similar in size to those growing on the control plate.

Experiment 4: Tubes of beef infusion broth were inoculated with *Staphylococcus aureus*, *E. coli*, or fresh saliva. To each tube was added a sufficient amount, by weight, of one of the brands of adhesive powder to give an approximate concentration of 10%. The tubes were incubated 24 hours. *Results:* The liquid broth was changed to a semi-solid consistency, as the result of the adhesive

[1] Received for publication November 25, 1944.

[2] Bartels, H. A., Microorganisms and tissue tolerance: Their Rôle in Prosthodontia. *J. D. Educ.*, **9**: 57, 1944.

powder absorbing moisture. Portions of this mucilaginous menstruum were removed and streaked on blood agar plates. Good growth of the various microorganisms inoculated were obtained.

DISCUSSION

The results of the above experiments indicate that the brands of adhesive powders tested do not contain substances inhibitory to bacterial proliferation. In view of the fact that certain individuals become sensitive to chemical agents even in minimal amounts, when exposed over long periods of time, it is questionable whether the incorporation in adhesive powders of substances to restrain bacterial growth, would be desirable.

CONCLUSION

Four brands of denture adhesive powders (Wernet's, Co-re-ga, Holdent, Fasteeth) were tested to determine if they possessed inhibitory qualities towards microorganisms. No evidence of such activity was obtained.

CHANGES IN THE WHITE CELL COUNT FOLLOWING TOOTH EXTRACTION IN ALBINO RATS AND RABBITS[1]

GEORGE J. ANDAY, M.D.,[2] AND RALPH L. FERGUSON, M.D.

Department of Medicine, Loyola University, School of Medicine, Chicago, Ill.

INTRODUCTION

The purpose of our work was to investigate to what extent tooth extraction influences the white blood cell count. Our attention was drawn to this problem by some of those cases of agranulocytosis in which the symptoms of the disease became apparent after tooth extraction. Hill (16) has already stated the close and, probably, direct connection between fatal agranulocytosis and tooth extraction immediately preceding it. Valuable reviews and observations on this subject have been published by Appleton (2), Cook (6), and Schaeffer (31). We also observed cases in which severe symptoms of agranulocytosis appeared immediately after tooth extraction.

To analyze these observations, we wanted to study the effect of tooth extraction, apart from the other oral symptoms so frequently met with in agranulocytosis. As a first step we wanted to know if tooth extraction has any effect upon the white cell count of healthy individuals. In the previously mentioned cases of agranulocytosis, the symptoms became apparent shortly after tooth extraction; therefore, the observation of white cell counts immediately after tooth extraction seemed of great importance to us. The white cell counts of healthy individuals was examined before and, again, at 15 to 20 minute intervals after tooth extraction. The examinations revealed that the white blood cell count dropped immediately after the extraction. This leukopenia was followed by moderate or marked leukocytosis.

The results, which will be published separately, were uniform in the first few cases.[3] As the number of patients increased, we had more cases which reacted differently. In considering these discrepancies, it was realized that we were confronted with many variants, such as difference in age, sex, race, and environment. An attempt was made to eliminate some of these. For this purpose, the effects of tooth extraction upon the blood of standardized animals was investigated. Following our experiments on normal animals, we set out to influence the function of the bone marrow by means of benzol, adenylic acid, and pentnucleotide, in some animals stimulating, in others retarding, leucopoiesis. Then we examined the influence of tooth extraction upon these animals.

METHODS AND MATERIALS

Seventy-four animals (51 albino rats and 23 rabbits) were used. They were considered the most suitable small laboratory animals, because their dental

[1] Read at a meeting of the Chicago section of the International Association for Dental Research, December 13, 1940. Received for publication November 27, 1944.

[2] Now in the A. U. S. Medical Corps.

[3] Drs. G. Le Mire and J. T. Coyle of the Mercy Loyola Clinics were most cooperative in these examinations.

development and morphology is well established (34, 29). All animals were females, and were kept under uniform conditions and diet. They were 6 to 7 months old at the time of experiment. The rats weighed 220 to 270 grams and the rabbits 2900 to 3800 grams.

The animals were given no food for 10 hours before and for 8 to 12 hours after tooth extraction. All experiments were carried out under ether anesthesia, which may not affect the white cell count, or may elevate it slightly. To observe normal fluctuations, blood cell counts were made 3 to 4 times prior to the experiments, and after 10 hours' fasting. After the operation, blood counts were made every ½ hour for 2 hours; later, at intervals of 1 to 2 hours and, finally, 8 to 24 hours for 3 to 6 days. Each time blood smears were taken and stained with Wright's stain for differential counts. In each case we extracted 1 lower incisor. The animals withstood the extractions well, and took food readily when it was given to them. Later, the overgrowing upper tooth caused some trouble, but, if this were cut off or extracted, the animal did well.

EXPERIMENTS

A. White Cell Count of Normal Albino Rat and Rabbit

The normal white cell count of rats has been studied by several investigators [see Donaldson (9)]. Scarborough (30) found, on 134 counts compiled from the literature, that the white cell count in normal rats showed an average of 11,590 per c.mm., with a normal range of 8,000 to 15,000. According to Crascoff, Fitz-Hugh and Farris (7), the normal white cell count in rats varies from 8,000 to 18,000 per c.mm. We observed an even greater variation in the white cell count of the different albino rats, rating from 6,000 to 20,000. The differential counts showed the characteristic lymphocytosis of the rat, the average normal values of differential counts being:

neutrophilic polymorphonuclears	26%
eosinophile polymorphonuclears	2%
basophile polymorphonuclears	1%
monocytes	3%
lymphocytes	68%

Many observations have been recorded on the normal white cell count of rabbits. Studer (37) found that the white cell count of normal rabbits varies from 6,600 to 11,200. Scarborough (30) collected 873 counts performed by 82 investigators and observed an average of 7,900 per c.mm., with a normal variation of 4,000 to 13,000 per c.mm. Haynes and Rigdon (15) in a statistical study found the mean leukocyte count for rabbits to be 10,662. In each individual animal we observed a variation from 5,000 to 12,000, the daily changes being greater in the rabbits than in the rats. In the differential counts of rabbits the polymorphonuclears (pseudoeosinophiles) are present in about the same proportions as the lymphocytes, namely:

polymorphonuclears	45%
eosinophiles	4%
basophiles	2%
monocytes	4%
lymphocytes	45%

B. The Effect of Tooth Extraction Upon the White Cell Count of Normal Animals

We observed that the tooth extraction in normal albino rats was immediately followed by leukopenia. The white cell count usually decreased within the first 30 minutes and reached its lowest level within 1 to 2 hours. It resumed its original level within 2 to 4 hours. This was followed by a leukocytosis which persisted for 12 to 24 hours, or even 48 to 72 hours. After that time, the white cell count returned to its initial level. The extent of leukopenia, and the degree and duration of leukocytosis varied in different animals. The degree of leukocytosis was seemingly independent from that of leukopenia which preceded it.

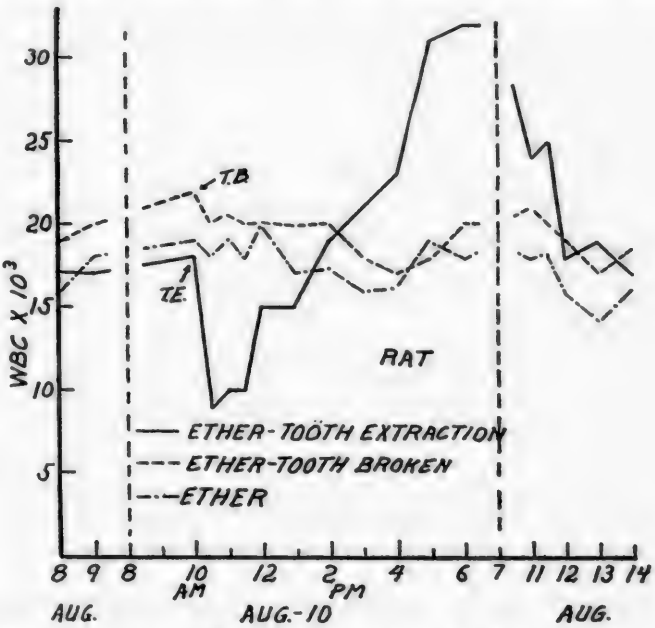

Fig. 1. Ether anesthesia, per se, did not influence normal variation in white cell count of one rat; nor was it affected by breaking off tooth under ether anesthesia of another. Tooth extraction in ether anesthesia of a third rat caused an immediate leukopenia followed by leucocytosis.

In some animals slight leukopenia was succeeded by marked leukocytosis of short or long duration. In other animals leukopenia was marked, and leukocytosis which followed it was only slight.

If a tooth was broken off, no change was observed in the white cell count (*fig. 1*). A linear incision of the skin, 2 inches in length and sewn with 6 stitches, caused an immediate slight elevation of the white cell count (*fig. 2*).

Differential counts revealed that in leukopenia the absolute number and percentage of polymorphonuclear leucocytes was decreased; whereas, during the subsequent leucocytosis, it increased in number (*fig 3*). At this stage, the

differential always showed a shift toward the less mature leukocytes containing band- and ring-shaped nuclei.

Identical experiments with similar results were carried out on rabbits.

C. The Effect of Tooth Extraction on the White Cell Count of Rabbits Treated With Benzol

Since the experiments of Selling (36), it is known that small doses of benzol, administered successively, have a depressing and, later, destructive effect upon the bone marrow of rabbits. Following the technique of Barta and Eros (3), we injected 1.0, 1.5, and 2.0 cc. pure benzol into the rabbits subcutaneously for 3 days. The result was a decrease in the white cell count, but the red cell count

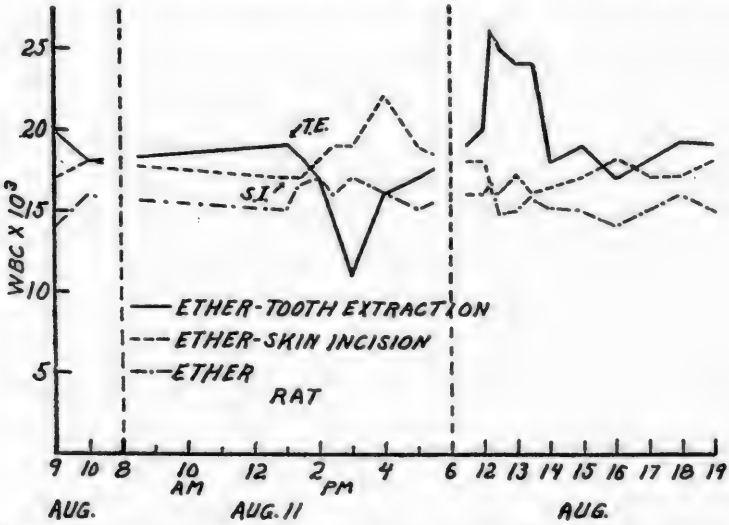

Fig. 2. Skin incision in ether anesthesia caused an immediate moderate leukocytosis, lasting for 4 hours. Tooth extraction on a second rat again caused leukopenia followed by leukocytosis which lasted for 48 hours. (See differential counts of this rat in *fig. 3.*)

and Hb content of the blood remained unchanged at the time of the experiment. After 3 days, the white cell count was below half its original level, and we then performed the tooth extraction on some of the animals. The result was an immediate decrease in the white cell count, which persisted for several hours. Later, it returned to the level of the previous count, but was not followed by leukocytosis. Leukopenia persisted for 1 to 2 hours; at that time, the control animals did not reveal significant changes.

In these cases, leukopenia was not succeeded by leukocytosis, although, at the time of the experiment, the bone marrow had the ability to produce it. This became evident by infecting some of these animals with massive doses of staphylococci, the reaction being marked leukocytosis up to 30,000 (*fig. 4*), 36,000 and 42,000 respectively.

D. The Effects of Tooth Extraction on the White Cell Count of Rabbits Treated With Adenylic Acid

Doan, Zerfas, Warren, and Ames (8) observed that intravenous administration of adenylic acid induces an immediate leukocytosis without previous leukopenia. In their experiments, leukocytosis persisted for 3 days, reaching its peak on the second day.

We used yeast adenylic acid in our experiments. One gram was dissolved in 10 c.cm. diluted normal sodium hydroxide solution (pH 6.6).[4] Intravenous administration of the solution was well tolerated but later the animals appeared sick, eating and moving less than the control animals, which had received the same amount of a buffer solution (10.0 cc.) of the same pH (6.6).

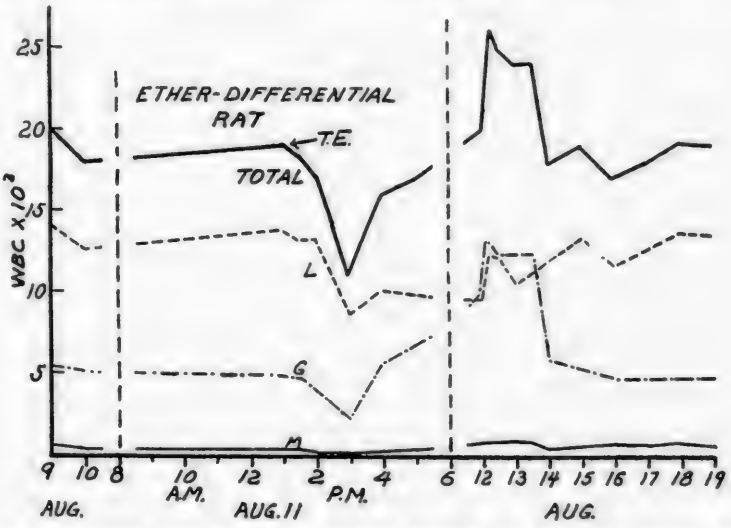

Fig. 3. Differential counts of rat whose total white cell count was indicated in *fig. 2*. This rat underwent tooth extraction under ether anesthesia. L—absolute number of leucocytes; G—absolute number of neutrophilic granulocytes, including those with band- and ring-shaped nuclei; M—absolute number of monocytes. During the leukopenia, granulocytes as well as lymphocytes show a decrease, whereas the subsequent leukocytosis is mainly due to an increase in the granulocytes. This included a greater number of the less mature forms.

Our experiments revealed that the adenylic acid caused a moderate leukocytosis without a preceding leukopenic phase, which set in 2 to 3 hours after the intravenous administration, persisting 3 to 4 hours. Then the white cell count fell to a lower level, close to normal. On the second day, a more marked second wave of leukocytosis was observed, lasting about 24 hours. On the third day, the number of white cells decreased sharply, and on the fourth or fifth day,

[4] The yeast adenylic acid used in our experiments was made available through the generosity of the Medical Dept. of Merck & Co. Inc., Rahway, N. J.

it again reached its original level. Rabbits, which were injected with buffer solution, showed no significant change beyond their normal fluctuations.

This leukocytic response to adenylic acid repeatedly showed similar results. Tooth extraction was performed when the first minor leukocytosis set in, that is, 2 hours after adenylic acid was injected. The result was an immediate and marked increase in the white cell count, reaching almost double the white cell count observed in rabbits which received adenylic acid but had no teeth extracted. This marked leukocytosis started on the first day, increased on the second day, and dropped to its original levels on the third to fifth day. From the second day, it paralleled that of the control animals (*fig. 5*).

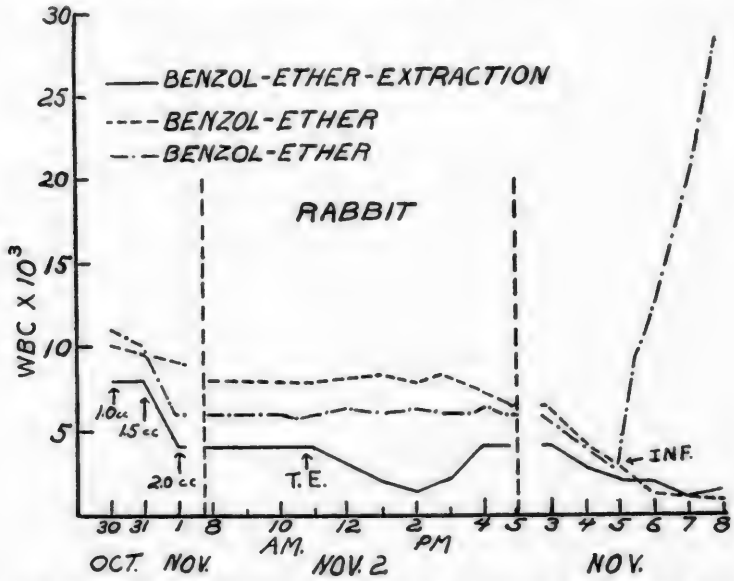

Fig. 4. In each of these 3 rabbits leukopenia was produced by increased amounts of benzol, administered subcutaneously on 3 successive days. On the fourth day tooth extraction was performed on one rabbit under ether anesthesia. This resulted in immediate leukopenia without subsequent leucocytosis. Other 2 rabbits underwent ether anesthesia only. In these 2 rabbits daily variations were smaller than usual. Three days later infection of one of these rabbits caused immediate very marked leucocytosis, with an appreciable 'shift to the left' in differential count.

In these experiments, tooth extraction caused an immediate marked leukocytosis without a previous leukopenic phase.

E. The Effect of Tooth Extraction On the White Cell Count of Rabbits Treated With Pentnucleotide

Pentnucleotide[5] (Jackson, Parker, Rinehart and Taylor (17)) is well known in the treatment of leukopenic conditions, especially agranulocytosis. In many such cases, it increases the white cell count in the peripheral blood. This drug

[5] We obtained the pentnucleotide through the courtesy of the Research Dept. of Smith, Kline & French Laboratories, Philadelphia, Pa.

is a mixture of sodium salts of pentose nucleotides and is obtainable in 10.0 cc. vials containing 0.7 g. of mixed nucleotides. If given intramuscularly to a normal individual pentnucleotide causes an immediate leukocytosis, generally, 6 to 10 hours after injection. Little or no 'shift to the left' was observed during the period of leucocytosis [Nordenson (26)]. In normal rabbits, intravenously injected with 4 to 6 cc. pentnucleotide, a transient leukopenia develops, usually lasting from 1 to 6 hours. Leukopenia is followed by a marked leukocytosis. A 'shift to the left' in all cases indicates that this leukocytosis is due to stimulation of the bone marrow.

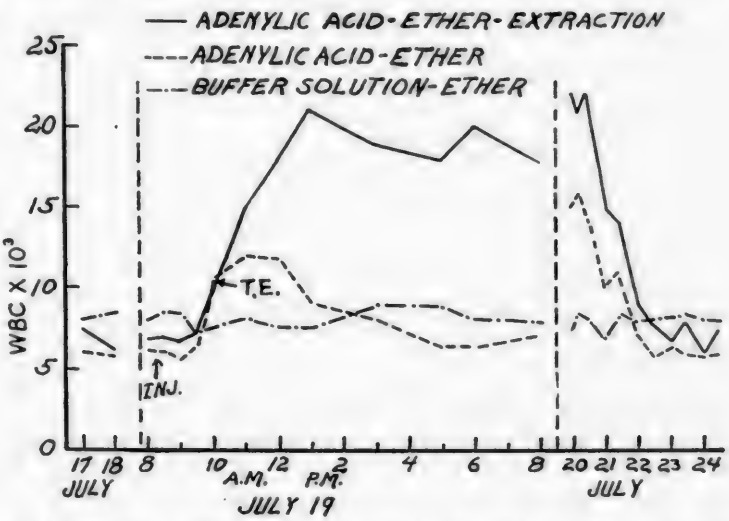

Fig. 5. Adenylic acid dissolved in 10 cc. diluted N. sodium hydroxide solution was given intravenously to 2 rabbits. Injection was followed by moderate and, next day, by marked leukocytosis. Tooth extraction on one of these rabbits caused immediate marked leukocytosis without previous leukopenia. Third rabbit received buffer solution intravenously and underwent merely ether anesthesia, and showed normal variations of white cell count

We injected rabbits intravenously with 6.0 cc. pentnucleotide and performed tooth extraction, in some animals at the stage of initial leukopenia, in others during the subsequent leukocytosis (*fig. 6*). The result was that tooth extraction in the first leukopenic stage caused a more marked decrease of the white cell count. Tooth extraction during the stage of leukocytosis caused a more prolonged leukocytosis than was observed in control animals. In this phase, tooth extraction emphasizes leukocytosis while there is no indication of a preceding leukopenia. During the period of leukocytosis, there is always a definite 'shift to the left'.

F. The Effects of Tooth Extraction Upon the White Cell Count In Splenectomized Albino Rats

Splenectomy of the albino rat is succeeded by a more or less severe anemia [see literature: Krumbhaar (19), Lauda (20), Marmorstain, Gottesman and

Perla (22)]. Notwithstanding, none of our animals were lost in the first 5 months after operation. The animals developed anemia and showed an increased number of reticulocytes, the appearance of normoblasts and, in some of the red blood cells, Howell-Jolly bodies. The anemia improved and the red cell count resumed almost normal levels within 4 to 8 weeks. The white cell count increased immediately after splenectomy and reached its maximum after 8 to 12 days. It gradually decreased and, over a period of 2 months, returned to almost the initial count prior to splenectomy.

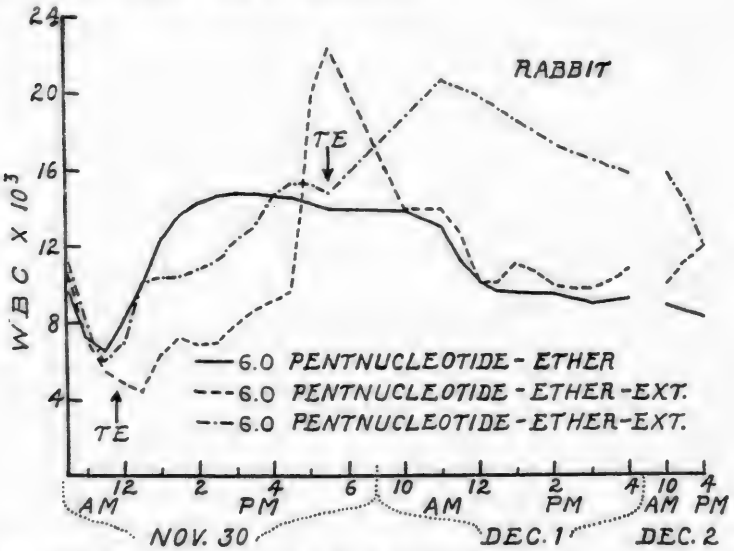

Fig. 6. Each of 3 rabbits received 6 cc. of pentnucleotide intravenously at 8.00 A.M. This resulted in leukopenia followed by leukocytosis. Tooth extraction performed at leukopenic stage in one animal furthered the leukopenia and accentuated, for a short time, the subsequent leukocytosis. Tooth extraction performed on second rabbit in stage of leukocytosis caused a more prolonged leukocytosis without previous leucopenia. Third rabbit which underwent ether anesthesia only indicates usual reaction of rabbits to pentnucelotide.

We first made 'acute' experiments, performing the splenectomy immediately before and after tooth extraction. We found, however, that the sudden changes in the white cell count following splenectomy did not render it feasible to evaluate the effects of tooth extraction. In the beginning, tooth extractions were performed 1 week after splenectomy, but later a 4-month interval was allowed.

1. Tooth extraction 1 week after splenectomy caused no appreciable changes in the white cell count. Table I illustrates a high initial white cell count in these animals. The tooth extraction is preceded and followed by a fluctuation in the white cell count. A similar fluctuation was observed in the white cell count of the control animals which were splenectomized at the same time and were subjected to ether anesthesia with the other animals, but had no teeth

extracted. Since normoblasts were present in large numbers in most of these animals, the white cell counts had to be corrected by the usual calculations. The figures in Tables I and II represent corrected values.

2. Tooth extractions performed 4 months after splenectomy resulted in changes in the white cell count similar to those observed in normal albino rats. The initial white cell count in these animals was normal or slightly above it.

TABLE I

Splenectomy was performed on each of these rabbits. One week later tooth extraction was carried out under ether anesthesia on 2 rabbits (L_1 and R_1). The 2 control animals (OO and LC_1) underwent ether anesthesia, but no teeth were extracted. The high white cell count shows irregular fluctuations on each of the animals, regardless of tooth extraction.

Date of extraction: 6/17/40. Date of splenectomy: 6/10/40

	9:00 A.M.	9:15 A.M.	9:45 A.M.	10:45 A.M.	12:15 A.M.	2:30 P.M.	6/18/40
L_1	51,000	T.E.	56,000	51,000	43,000	50,000	42,000
R_1	53,000	T.E.	45,000	52,000	48,000	42,000	39,000
OO (Control)	42,000	—	39,000	33,000	32,000	35,000	47,000
LC_1 (Control)	64,000	—	64,000	54,000	53,000	52,000	54,000

TABLE II

Splenectomy was carried out on each of these rabbits. Four months later tooth extraction was performed under ether anesthesia on 2 rabbits (L_2 and OO). The 2 control animals (L_2R_c and UL_2) underwent ether anesthesia but no teeth were extracted. The tooth extraction in the 2 rabbits caused transient leukopenia followed by leukocytosis, similar to the reaction of normal animals. The control animals show negligible changes in their white cell count.

Date of extraction: 8/4/40. Date of splenectomy: 4/7/40

	8:00 A.M.	8:30 A.M.	9:00 A.M.	9:30 A.M.	10:00 A.M.	11:00 A.M.	1:00 P.M.
L_2	18,000	T.E.	16,000	11,700	12,000	15,400	24,000
OO	22,000	T.E.	14,000	15,200	18,000	19,000	28,000
L_2R_c (Control)	24,000	—	22,200	24,000	21,200	22,600	22,000
UL_2 (Control)	19,800	—	21,000	19,200	19,000	19,000	20,000
	3:00 P.M.	6:00 P.M.	8/5/40	8/6/40	8/7/40		
L_2	30,000	36,000	29,300	27,000	20,000		
OO	29,000	29,200	24,500	20,000	21,000		
L_2R_c (Control)	21,800	23,000	22,000	24,000	21,400		
UL_2 (Control)	21,000	22,000	20,000	18,400	22,000		

The tooth extraction caused a decrease in the white cell count which was succeeded by leukocytosis within a few days. Table II illustrates the results of one of our experiments.

DISCUSSION

For the proper evaluation and understanding of our observations, we have to revert to the basic problems of leukocytosis. The reaction of leukocytosis,

with previously normal white cell formation, may appear as one of 2 types: 1. Immediate leukocytosis; or 2. Leukocytosis, preceded by leukopenia. Both types have been frequently reported in the literature, of which the following are examples:

An *immediate leukocytosis* occurs most frequently in infections produced by pyogenic organisms. It has been observed after i.m. injection of milk (Kracke, 18); after operations of individuals (Kracke, 20), as well as experimental animals (Schulz, 35); after subcutaneous administration of nucleic acid in dogs (Ames and Huntley, 1); after intravenous injection of adenine and guanine nucleotides, adenylic acid in rabbits (Doan et al., 8); and in insulin shock in dogs (Muller and Petersen 25).

Leukocytosis, preceded by leukopenia is, likewise, well known. Lowit (21) observed it after injection of bacterial proteins, and peptones or urates. He was so much impressed by the leukopenia which preceded the leukocytosis that he came to the conclusion that the 2 stages were firmly associated, and that leukocytosis was called forth by the leukopenia which precedes it. Similarly, leukocytosis, preceded by leukopenia was observed after the injection of extracts of bone marrow, thymus and spleen into animals (Goldscheider and Jacob, 14); after subcutaneous injection of dead typhoid and coli bacilli into animals (Studer, 37); after injection of *B. pyocyaneus* into animals (Ewing, 11); after injection of pulverized carbon and dyes into experimental animals (Kracke, 18); after intravenous injection of dead bacteria into rabbits (Wells, 38); after intravenous injection of sodium nucleinate into rabbits (Doan, Zerfis, Warren and Ames, 8); after intravenous injection of salvarsan into susceptible individuals (Muller, 24); in the state of chill produced by intravenous injection of *B. coli* into dogs (Petersen and Muller, 28); and the chill of therapeutically induced malaria infection in cases of general paresis (Muller, 24). Menkin (23) studied a leukocytosis promoting factor of exudates. He observed that in animals intravascular injection of serum or exudate causes leukocytosis, preceded by leukopenia.

The *analysis of the mechanism of leukocytosis, preceded by leukopenia*, is elucidated in the papers of Wells (38), Muller and Petersen (25) and Doan, Zerfas, Warren and Ames (8) which present original observations as well as references to earlier literature. During the first leukopenic stage, the large numbers of white blood cells are withdrawn from the peripheral circulation and accumulate in the capillaries of internal organs. According to Wells (38), these internal organs are chiefly the spleen and liver; according to Muller and Petersen, the pancreas, stomach and gastro-intestinal tract are also involved. On the other hand, Doan, Zerfas, Warren and Ames (8) found that in the sodium-nucleinate induced leukopenic and leukocytic state the spleen is solely responsible for the first, immediate leukopenic stage and that this could be averted by splenectomy. However, in the experiments of Zeckwer (39) splenectomy did not prevent the leukopenia following the intravenous injection of sodium nucleinate. Similarly, in Faludy's (13) experiments, the leukopenia, following intravenous injection of *B. coli* vaccine, was not influenced by splenectomy. In our experiments on albino rats the changes of the white cell count after tooth extraction, 4 months after splenectomy, were similar to that of the normal, non-splenectomized animals. Therefore, it is probable that the other organs, indicated by Wells (38) and Muller and Petersen (25) retained the white blood cells.

An *analysis of the second stage, that is leukocytosis*, is more definite. It originates, evidently, from the bone marrow as indicated by the appearance of less mature white blood cells in the peripheral blood during leukocytosis.

The data derived from our own experiments can be divided into 2 groups: (1) tooth extraction on animals with previously normal white cell formation, and (2) tooth extraction on animals whose white cell formation has been influenced by preceding treatment.

In group 1 (normal animals) it was found that tooth extraction caused a

leukocytosis preceded by leukopenia; whereas, abdominal surgery or skin incision induced an immediate leukocytosis without preceding leukopenia. It is difficult to explain why tooth extraction causes a leukocytosis which differs from that following surgery of the abdomen or incision of the skin. The close proximity of the bone marrow to the alveoli may be responsible for this distinction. The jaw contains active red marrow, as well as aplastic yellow marrow. Its distribution under normal and pathologic conditions was studied by Schonbauer (32, 33) and Box (4, 5) who made extensive observations on human jaws, as well as studies of animal experiments.

The possibility arises that a minute lesion of the bone marrow can elicit changes in the white cell count. We believe that such small lesions are caused, for example, by sternal puncture which is performed in routine hematologic examination. In some of our cases sternal puncture actually elicited leukocytosis preceded by leukopenia. Nevertheless, the effects of a transient bacteremia after tooth extraction, as first reported by Okell and Elliott (27), may also be considered.

The leukocytic response after tooth extraction in animals whose white cell formation has been influenced by preceding treatment (group 2) varies markedly from that of normal animals. The reaction is either leukocytosis or leukopenia, depending upon the state of the white cell formation. This is illustrated in the benzol experiment in which tooth extraction accentuates the prevailing leukopenia, and in the adenylic acid experiments in which it furthers the leukocytosis. This is also illustrated in our pentnucleotide experiment in which tooth extraction increased leukopenia in the initial stages. If performed some hours later, it promotes a leukocytosis.

Further observations on human material will show to what extent the results of our animal experiments can be applied to man. Our observations on dental patients at the Loyola University Clinics are not as yet completed. Faillo (12) studied the changes in the white cell count on 20 patients before and after tooth extraction. His report is encouraging and our results seem to confirm his work. For those cases of agranulocytosis immediately preceded by tooth extraction, our experiments may offer a solution of the problem. Unrecognized damage to the white cell formation may be aggravated if tooth extraction is superimposed. This mechanism would be similar to that observed in our experiments in teeth that were extracted in benzol-treated animals. Further careful clinical observations are necessary to decide this.

SUMMARY

1. Tooth extraction has an immediate effect upon the white blood cell count of albino rats and rabbits.

2. This effect varies in animals with normal, stimulated, or depressed white cell formation. In normal animals, both in albino rats and rabbits, tooth extraction results in an immediate transient leukopenia followed by leukocytosis. Skin incision is followed only by immediate moderate leukocytosis.

3. In rabbits treated with benzol for a brief period, the immediate reaction

to tooth extraction is a still deeper depression of the white cell count, which is not followed by leukocytosis.

4. In rabbits treated with adenylic acid, the reaction immediately after tooth extraction is a leukocytosis which is more marked than in control animals, without any previous decrease in the white cell count.

5. In rabbits treated with pentnucleotide, the tooth extraction causes a more pronounced leukopenia if performed in the leukopenic stage, and a more pronounced leukocytosis if carried out in the stage of leukocytosis, as compared to that of the control animals.

6. In splenectomized albino rats, the results of tooth extraction vary in the time which elapses between splenectomy and tooth extraction. Albino rats, which are splenectomized shortly before tooth extraction, show no appreciable reaction. If tooth extraction is performed about 4 months after splenectomy, it causes a marked leukocytosis preceded by a moderate leukopenia, similar to the reaction of normal, non-splenectomized rats.

We are greatly indebted to Dr. B. Orban, for his generous assistance and valuable suggestions, and to Drs. C. L. F. Birch, J. P. Weinmann, I. Schour, and H. Sicher for their advice. Miss H. E. Reynolds, M. T. gave us valuable technical help in our experiments.

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STUDIES ON THE FERMENTATION OF SORBITOL BY ORAL MICROORGANISMS[1,2]

THOMAS C. GRUBB, PH.D.

University of Maryland, School of Dentistry, Baltimore, Md.

INTRODUCTION

The most generally accepted conception of dental caries etiology at present is that certain oral microorganisms ferment carbohydrates on the tooth surface producing sufficient acid to decalcify the enamel. The microorganisms most frequently implicated are the lactobacilli (1) and streptococci (2) because they are so frequently isolated from carious teeth, and are both aciduric and acidogenic. It is not proposed to discuss which is the more important for there is good evidence implicating both genera; and, quite possibly, both may be equally involved.

On the basis of this conception of caries etiology, modern investigators have introduced a number of substances tending to inhibit or prevent microbic fermentation in the mouth. It is believed that such substances as sodium fluoride, synthetic vitamin K, iodoacetic acid, etc. block various enzymic reactions produced by acidogenic bacteria and thus the end products, acids, are not formed. The introduction of such substances for the prevention or control of caries is indeed a logical procedure but sufficient time has not elapsed to judge adequately the value of these measures. The purpose of this report is to present another method of attacking the problem of caries prevention and control. Whereas previous methods have sought to restrict or inhibit the action of microorganisms on carbohydrates, the method introduced here consists of substituting a sugar-alcohol which is but slowly attacked by acidogenic organisms. This new approach to the problem was suggested by one of our colleagues[3] on the basis of the following reasoning.

Bergey's Manual of Determinative Bacteriology (3) states that sorbitol is not fermented by *Lactobacillus acidophilus*. Since *L. acidophilus* is thought to be one of the most important causes of caries, and since sorbitol is sufficiently sweet to be substituted for sucrose in most instances where the latter is now used for sweetening purposes it was logical to believe that the substitution of this sugar-alcohol for sucrose or glucose would reduce or prevent acid production in the mouth. Although a preliminary study of the problem showed that one of the premises in our reasoning was incorrect for the literature reveals many

[1] Received for publication December 11, 1944.

[2] Now with Vick Chemical Co., Flushing, N. Y.

[3] The writer is indebted to Dr. John C. Krantz, Jr., Professor of Pharmacology, School of Medicine, University of Maryland, for initiating this study and for much valuable help during its progress.

instances where large numbers of *L. acidophilus* strains have fermented sorbitol (4, 5, 6), it was decided to undertake the investigation because of the following discovery.

In the first tests made on the fermentation of sorbitol by oral bacteria it soon became evident that sorbitol was fermented much more slowly than sucrose or dextrose. The final pH of the medium in which sorbitol was fermented was always higher than the pH of media containing sucrose or dextrose. The possibilities of sorbitol as a substitute sugar became more promising when it was found that in 90% of the samples, the pH of the medium in which sorbitol had been fermented by oral bacteria was above the range usually considered necessary to cause enamel decalcification (pH 5.0–5.5). Furthermore, since Fosdick, Campaigne and Fancher (7) have shown that a major factor in caries production is the *rate of acid formation*, the use of a sugar substitute which would appreciably retard the rate of acid formation should aid in the prevention or control of caries. It thus appeared that a thorough investigation of the action of oral microorganisms on sorbitol should prove of considerable interest; and it is the purpose of this report to describe the results of this phase of the investigation. The clinical studies on the use of sorbitol as a substitute sugar are in progress and will be reported on subsequently.

Before proceeding with a consideration of the experimental work, it is believed of interest to outline briefly the history of sorbitol which for so many years has been classed as a "rare sugar." In 1852, Pelouze (8) described the preparation of sorbine from the berries of mountain ash. He found sorbine to be a crystallizable sugar-alcohol "resisting alcoholic fermentation." Knowledge of sorbitol was greatly increased by the contributions of Bertrand (9), in 1904, who not only studied the chemical properties of sorbitol, but also described the preparation of sorbose by bacteriological methods. He found that sorbitol was present in many fruits and vegetables beside mountain ash berries, and confirmed Pelouze's observation that sorbitol resisted fermentation by beer yeasts.

For many years sorbitol was indeed a "rare sugar" because its difficulty of preparation made it expensive and it found little usage outside of its employment by bacteriologists for fermentation tests in the identification of various bacteria. Recently, however, it has become commercially practicable to produce vast quantities of sorbitol by the electrolytic reduction of glucose, thereby lowering the cost of sorbitol to such an extent that although it is more expensive than sucrose or dextrose, it now becomes economically sound to consider sorbitol as a possible substitute sugar for sweetening foods, beverages, confections, etc. The humectant properties of sorbitol make it an excellent plasticizer for adhesives, glues, gelatins, etc. Large quantities of sorbitol are being used in the synthesis of ascorbic acid.

The physical, chemical and pharmacologic properties of sorbitol are favorable for its use as a sweetening agent. Sorbitol is said to be approximately one half as sweet as sucrose. In recent years a number of studies (10, 11, 12) on the use of sorbitol as a substitute sugar for diabetics have been published which

indicate that it has no toxic effects when consumed in reasonable amounts. Some investigators (12) have found that only when a dose of 50 grams or more is consumed at one time by the average adult, slight catharsis may be expected. The physical and chemical characteristics of sorbitol lend themselves well to the substitution of this sugar-alcohol for sucrose or dextrose wherever the latter are used for sweetening purposes. There is therefore every reason to believe that sorbitol could be easily substituted for the common sweetening agents in the prevention or control of caries.

EXPERIMENTAL

Quantitative Estimation of Sorbitol-Fermenters in Saliva

At the beginning of this investigation it was believed important to determine the percentage of individuals whose saliva contained sorbitol-fermenting microorganisms; and to determine the concentration of these organisms in the saliva. In this way it was thought that if the proportion of individuals was found whose saliva did or did not contain sorbitol-fermenters, the usefulness of sorbitol in caries prevention could be quickly estimated.

In the first experiments, the saliva samples were streaked on nutrient agar (pH 7.0) containing 1% sorbitol[4] with brom thymol blue as the indicator; and on tomato juice agar (pH 6.0) containing 1% sorbitol with brom cresol green as the indicator. These plates were incubated 48 hours at 37°C. and the percentage of acidogenic colonies determined. This procedure was unsatisfactory for both the nutrient agar and tomato juice control plates without added sorbitol contained a sufficient amount of fermentable substances to mask the action of the organisms on sorbitol. To eliminate these substances, the media were first inoculated with *Saccharomyces cerevisiae*, incubated at 37°C. for 24 hours, filtered and then sterilized before the sorbitol and indicator were added. While this procedure eliminated the interfering fermentable substances, it also deprived the media of much of their nutritive properties as indicated by an approximately 70% reduction in the number of colonies found on the plates of media so treated. About this time, a new dehydrated medium became available, Trypticase (Baltimore Biological Laboratories), which contains no fermentable carbohydrates and provides excellent growth-promoting properties for many fastidious organisms, including *L. acidophilus* and streptococci.

A medium was prepared containing 2% Trypticase, 2% agar, 1% sorbitol and divided into 2 lots, one being adjusted to pH 7.0 and containing brom thymol blue, and the other adjusted to pH 6.0 and containing brom cresol green. These media were sterilized at 15 pounds for 15 minutes and plates poured. Saliva samples from 50 persons were each diluted 1:1000, 1:10,000 and 1:100,000 in sterile water and 0.1 cc. of each dilution was streaked on the pH 7.0 and 6.0 plates with sterile glass spreaders. After the plates had been incubated at 37°C. for 48 hours the acidogenic and non-acidogenic colonies were differentially counted. About 28% of the saliva samples did not contain any sorbitol-

[4] The sorbitol used in this investigation was furnished through the courtesy of the Atlas Powder Co., Wilmington, Del.

fermenters, while in those saliva samples containing fermenters, the counts ranged from 80,000 to 121,000,000 per cc. of saliva. However, it is not believed that these results give a true representation of the presence of sorbitol-fermenters in saliva for the following reasons: (a) Many of the colonies which fermented sorbitol on the plates did not ferment this sugar when transferred to a broth medium containing sorbitol. (b) Many yellow chromogenic colonies were unavoidably mistaken for acidogenic colonies in counting the plates, the chief offender being an organism resembling *Neisseria flava*. (c) Some colonies fermented the proteins in the medium to a sufficient degree to change the color of the indicator. Bozeman and Orcutt (13) have recently shown that acid is produced from the peptones in a medium containing a fermentable carbohydrate. (d) Saliva, which did not show any sorbitol-fermenters in the plate counts, always fermented sorbitol when the saliva was inoculated into broth tubes containing this sugar-alcohol.

For these reasons it would appear that little or no significance can be attached to this attempt, using standard bacteriologic procedures, to estimate the relative number of sorbitol-fermenters in saliva. Although these results are of a negative value so far as this investigation is concerned, they are included in the report in order to illustrate the difficulties involved in making differential counts of acidogenic bacteria by the usual methods, and in the hope that this experience may prove of value to others who may plan similar experiments. Since the value of sorbitol as a possible caries control agent lies principally in the fact that it is fermented much more slowly than dextrose or sucrose, as will be shown in the following section, it makes little difference whether or not the individual's saliva contains sorbitol-fermenting organisms, and, therefore, no further attempt was made to devise a satisfactory technique for making a differential fermentation count.

Rate of Sorbitol Fermentation in Aerobic Cultures

In order to determine the rate and degree of fermentation of sorbitol in aerobic cultures by oral microorganisms, samples of saliva were collected from 122 individuals, including those with active caries and caries resistant individuals. Saliva flow was stimulated by chewing sterile paraffin and the saliva collected in sterile tubes. In most cases, the saliva was immediately inoculated into the test media, while a few samples were held 12–18 hours in the refrigerator before inoculation. One drop of saliva was inoculated into 10 cc. of nutrient or Trypticase broth (pH 7.4) containing M/10 concentrations of sorbitol, dextrose or sucrose. A similar inoculum was placed in broth without any sugar to serve as a control. All carbohydrate media used in this phase of the investigation were sterilized by Seitz filtration to avoid any possible hydrolysis of the sugars. Four tubes of each sugar and control were inoculated with the saliva from each individual and the tubes placed in the 37°C. incubator. At intervals of 3, 6, 12, and 24 hours, a tube of each sugar and the control broth were removed from the incubator and the pH and titratable acidity determined on the same sample. The pH determinations were obtained with a glass electrode in a Beckman pH

meter; and the titratable acidity was found by the addition of N/40 NaOH using phenolphthalein as the indicator. The average or mean results of the pH determinations are shown in Table I.

Table I clearly shows that sorbitol is fermented under aerobic conditions much more slowly than either sucrose or dextose. With the exception of the difference between the pH of sorbitol and sucrose and dextrose after 3 hours, and sorbitol and dextrose after 6 hours, all other differences are statistically significant, as explained in the footnote of Table I. It is to be noted that the pH of sorbitol, even after 24 hours incubation, does not fall within the pH range required for enamel decalcification, while at the end of 12 hours incubation both sucrose and dextrose are well below this range. *Fig. 1*, a graph plotted on semi-logarithmic

TABLE I

Mean pH values of the broth control, sorbitol, dextrose, and sucrose broths inoculated with saliva and incubated aerobically at 37°C.

TIME	BROTH CONTROL	SORBITOL	DEXTROSE		SUCROSE	
	mean pH	mean pH	mean pH	S.E.* Diff.	mean pH	S.E.* Diff.
Before inoculation	7.4	7.4	7.4	—	7.4	—
3 hours	7.1	7.1	6.9	*0.29*	7.0	*0.35*
6 hours	7.0	6.9	6.4	*0.52*	5.8	0.32
12 hours	6.8	6.8	4.7	0.88	4.5	0.88
24 hours	7.0	6.5	4.5	0.27	4.4	0.29

* The standard error of difference is derived from the following formula: $\sqrt{\frac{(S.D)^2}{N_1} + \frac{(S.D)^2}{N_2}}$. If twice the standard error of difference is greater than the difference between 2 observed means, the difference between the observed means is not considered statistically significant. In the above table the standard error of difference has been computed between the mean pH values of sorbitol and each of the other 2 sugars. Those standard errors of difference, which when doubled, are greater than the difference between two observed means, are italicized in the above table.

paper, shows the relative changes in pH produced in the various sugar and control broths during the 24-hour incubation period.

Since the higher pH value produced by sorbitol broth after 12 and 24 hours incubation is statistically significant, there can be little doubt that this sugar is fermented much more slowly than the other 2 sugars studied. Furthermore, a frequency distribution chart of the 122 salivas studied showed that only 6.6% of the saliva samples inoculated into sorbitol broth reached the critical pH level of 5.5 or lower after 24 hours incubation and none fell below pH 5.0 as shown in *fig. 2*.

The amount of acid formed in the sugar broths inoculated with saliva was determined by titration with NaOH, as previously described. The average or mean titration values are shown in Table II.

As would be expected from the results of the pH determinations, the titratable acidity in the sorbitol broth was lower than in either of the other 2 sugars.

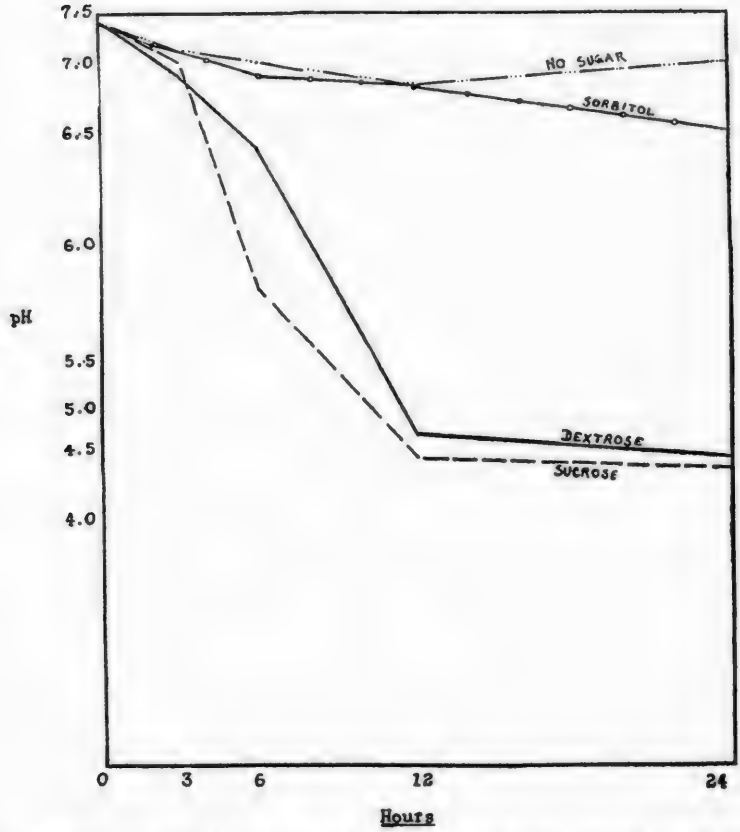

Fig. 1. Mean pH values of broth control, sorbitol, dextrose, and sucrose broths incubated aerobically at 37°C. for 3, 6, 12 and 24 hour intervals.

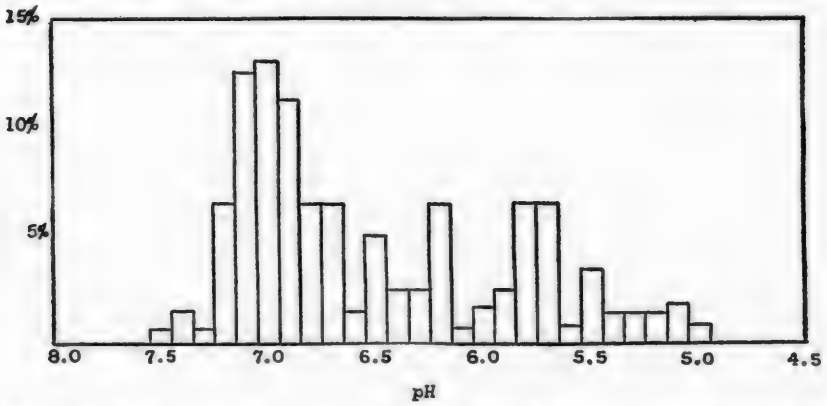

Fig. 2. Showing percentage distribution of pH values of sorbitol broth inoculated with saliva and incubated aerobically at 37°C. for 24 hours.

TABLE II

Mean titratable acidity values of the broth controls, sorbitol, dextrose, and sucrose broths inoculated with saliva and incubated aerobically at 37°C.

TIME	BROTH CONTROL	SORBITOL	DEXTROSE		SUCROSE	
	mean NaOH	mean NaOH	mean NaOH	S.E.* Diff.	mean NaOH	S.E.* Diff.
	cc.	*cc.*	*cc.*		*cc.*	
Before inoculation	3.0	3.0	3.0	—	3.0	—
3 hours	5.6	6.2	6.1	*0.68*	6.2	—
6 hours	6.2	6.6	8.5	0.64	9.1	0.68
12 hours	7.9	7.8	13.9	2.26	12.4	1.91
24 hours	8.3	9.0	17.0	1.35	16.5	1.28

* See note in Table I for use and significance of standard error of difference.

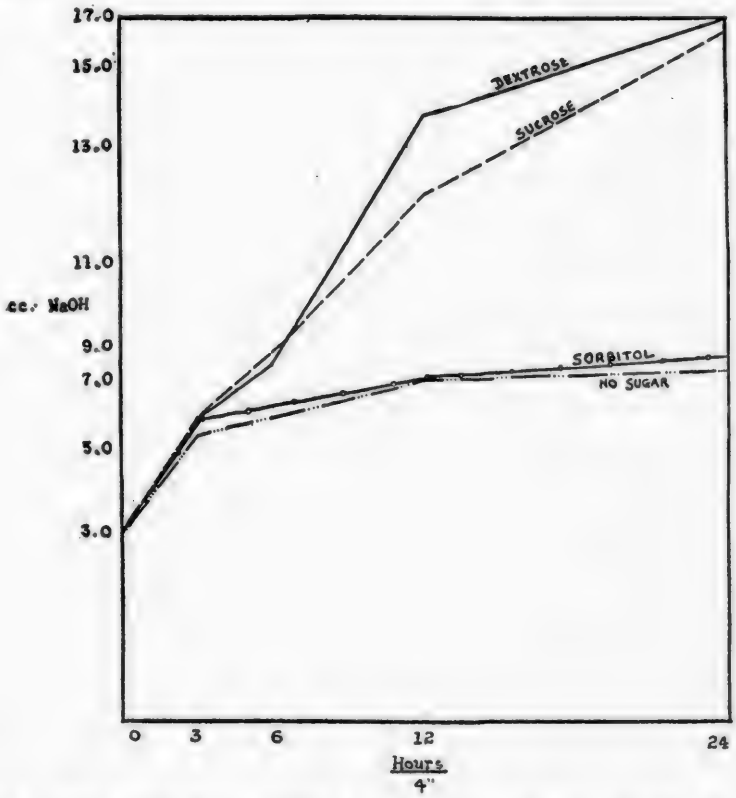

Fig. 3. Mean titrable acidity values of broth control, sorbitol, dextrose, and sucrose broths incubated aerobically at 37°C. for 3, 6, 12, and 24 hours.

With the exception of the 2 instances noted, the differences in acidity were statistically significant. *Fig. 3*, a graph plotted on semi-logarithmic paper,

represents the changes in titratable acidity produced by the three sugars and control broth.

It seems evident both from the pH determinations and the titratable acidity values observed in the broth containing the 3 sugars inoculated with saliva and incubated aerobically at 37°C. that sorbitol is fermented much more slowly than dextrose or sucrose. Since these figures represent the mean or average changes, it is important to note that only 6.6% of the sorbitol broths reached a pH of 5.5 or lower. Although these results are based on the observation of only 122 samples, the fact that twice the standard error of difference between sorbitol and the other 2 sugars was less than the observed difference in most tests makes it extremely unlikely that if a greater number of samples were tested, the results would differ appreciably.

Rate of Sorbitol Fermentation in Anaerobic Cultures

Since it is quite possible that some of the fermentative changes involved in the production of acid on the enamel surface occur under anaerobic conditions, it appeared important to investigate the action of oral microorganisms on the test sugars under anaerobic culture conditions. For this purpose a modified thioglycollate medium was prepared containing an amount of sodium thioglycollate in excess of that used in Brewer's original formula (14) in order to inhibit the growth of aerobic organisms so far as possible. However, aerobic organisms did grow in this modified medium so that both aerobic and anaerobic organisms were cultured simultaneously. This would appear to simulate the conditions on the enamel surface where both aerobic and anaerobic fermentations undoubtedly take place concurrently. The composition of the medium was as follows: Nutri peptone 5 gms., NaCl 2.5 gms., agar 0.25 gms., sodium thioglycollate 0.5 gms., and 0.1 cc. of a 1% solution of methylene blue, all dissolved in 1000 cc. of distilled water. To this basic medium M/10 concentrations of the test sugars were added and the pH adjusted to 7.4. The medium was tubed in 10 cc. quantities and autoclaved at 10 lbs. for 20 minutes, since it was not possible to sterilize this medium by Seitz filtration due to the presence of the agar. The inoculation of this medium and the determination of the pH were carried out in the same manner as previously described for the aerobic cultures. The titratable acidities were not determined because the color of the medium made it impossible to judge accurately differences in the color changes of phenolphthalein. The results of testing 55 samples of saliva are shown in Table III.

The results of this experiment are similar to those obtained with the aerobic cultures with the exception that the pH did not fall quite so low. Again it is evident that sorbitol is fermented more slowly than either of the other 2 sugars. The statistical analysis of the results shows that with the exception of sucrose after 3 hours incubation, the differences between the pH values of sorbitol and the other sugars are significant. The changes in pH in the various broths during the 24-hour incubation period are shown in *fig. 4*, plotted on semi-logarithmic paper.

TABLE III

Mean pH values of thioglycollate broth containing sorbitol, dextrose, and sucrose inoculated with saliva and incubated at 37°C.

TIME	BROTH CONTROL	SORBITOL	DEXTROSE		SUCROSE	
	mean pH	mean pH	mean pH	S.E.* Diff.	mean pH	S.E.* Diff.
Before inoculation	7.3	7.3	6.9	—	7.2	—
3 hours	7.3	7.2	6.6	0.27	7.0	0.27
6 hours	7.3	7.1	5.6	0.36	5.8	0.39
12 hours	7.3	7.1	5.1	0.50	5.1	0.54
24 hours	7.5	6.9	4.9	0.31	4.9	0.31

* See note in Table I for use and significance of standard error of difference.

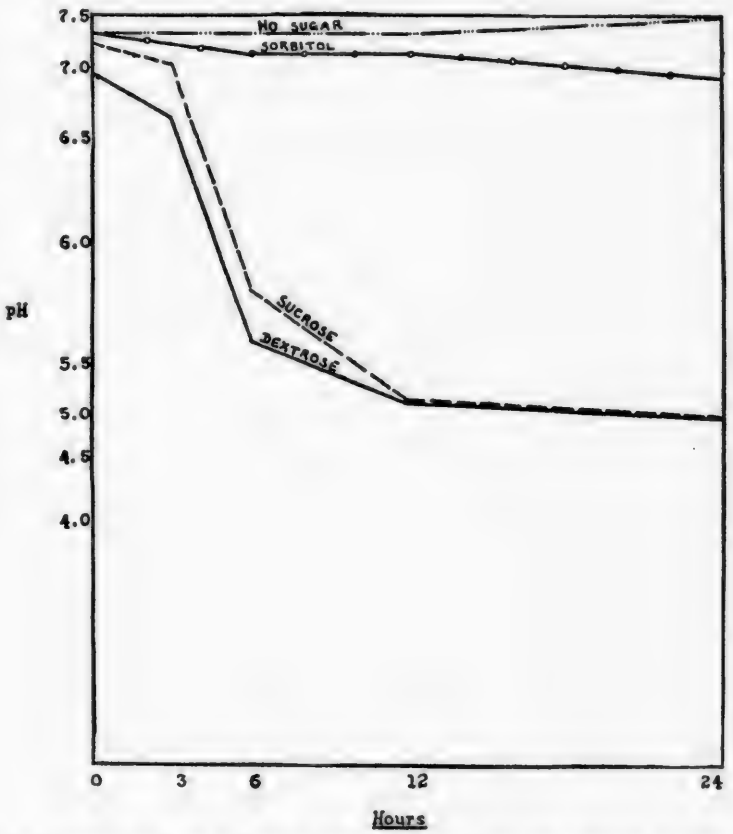

Fig. 4 Mean pH values of broth control, sorbitol, dextrose, sucrose in thioglycollate media incubated at 37°C. for 3, 6, 12 and 24 hour intervals.

Rate of Sorbitol Fermentation by L. acidophilus, Streptococci, and Yeasts

In recent years many investigators have contended that caries is initiated by the acid production of specific organisms, particularly *L. acidophilus* and the streptococci. Assuming that such an hypothesis is correct, it becomes important to learn how rapidly these organisms will attack sorbitol as compared with their action on dextrose and sucrose under similar conditions. Since the rôle of yeasts is as yet unevaluated in the production of caries and since Fosdick and Starke (15) have shown that the fermentative properties of *L. acidophilus* are greatly enhanced by the presence of yeasts, it also appeared of interest to determine the rate of sorbitol fermentation by yeasts growing alone and together with *L. acidophilus*.

In the following study 16 strains of *L. acidophilus* were used. Eight strains were recently isolated from carious teeth, while the remainder were stock cultures obtained from other laboratories.[5] The 4 streptococci and 15 yeast strains were freshly isolated from saliva. Cultures of the yeasts on cornmeal agar and Sabourad's dextrose agar indicated that 7 were *Monilia sp.*, 3 *Torula sp.* and 5 unidentified. In the 12 mixed cultures of yeasts and *L. acidophilus*, 10 were the original mixtures found in saliva, 5 of the yeasts being *Monilia* and 5 *Torula*. The remaining 2 were artificially mixed, one with a *Torula* and one with a *Monilia* strain. It is interesting to observe that in attempting to obtain pure cultures of streptococci, *L. acidophilus* and yeasts by streaking saliva on tomato juice agar, little difficulty was experienced in separating the streptococci from the other organisms, but invariably the yeasts and *L. acidophilus* literally clung to each other making repeated streakings necessary before pure cultures were obtained. This observation gains significance in the light of Fosdick and Starke's finding (15) that yeasts supply phosphatase which aids in the growth of acidophilus organisms.

The pure or mixed cultures of the above organisms were grown in Trypticase broth for 24 hours at 37°C. and 1 loopful was inoculated into Trypticase broth (pH 7.4) containing M/10 concentrations of sorbitol, dextrose or sucrose and into the control broth without sugar. The cultures were incubated at 37°C. and the pH and the titratable acidity determinations made as described in the preceding sections. The results of these determinations are shown in Tables IV and V.

The pure or mixed cultures of the inoculated organisms also failed to ferment sorbitol as rapidly as sucrose and dextrose. Again it was observed that the pH of sorbitol broth at the end of 24 hours incubation was well above the range where enamel is decalcified. These results also confirm Fosdick's observation regarding the fermentative powers of mixed cultures of yeasts and *L. acidophilus*, for it will be noted that the mixed cultures produced a lower pH on dextrose and sucrose than either organism by itself.

In a preceding section the reasons for studying the anaerobic fermentation

[5] The writer is indebted to Dr. J. R. Blayney of the University of Chicago for the cultures of *L. acidophilus* (Hadley types I, II, and III) and to Dr. R. P. Tittsler of the U. S. Department of Agriculture, Bureau of Dairy Industry, for cultures of these organisms.

of the test sugars were outlined and it likewise seemed advisable to determine the action of the pure and mixed cultures on the test sugars cultured anaerobically. The methods employed for the anerobic cultivation were the same as those described when saliva was inoculated. The pH and titratable acidity determinations are given in Tables VI and VII.

The pH and titratable acidity values obtained by culturing in the thioglycollate medium are similar to those obtained by aerobic cultivation except that

TABLE IV

Mean pH determinations of broth control and sugar broths inoculated with various organisms and incubated aerobically

TIME	L. ACIDOPHILUS				STREPTOCOCCI				YEASTS				YEAST AND L. ACIDOPHILUS			
	N.S.*	Sorb.	Dext.	Suc.	N.S.	Sorb.	Dext.	Suc.	N.S.	Sorb.	Dext.	Suc.	N.S.	Sorb.	Dext.	Suc.
Before inoculation	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4
3 hours	7.2	7.2	6.6	7.2	7.3	7.3	6.7	7.2	7.3	7.4	7.1	7.4	7.2	7.2	6.8	6.6
6 hours	7.2	7.1	6.1	7.1	7.3	7.3	6.3	6.7	7.3	7.4	6.7	7.2	7.0	6.8	5.6	6.2
12 hours	7.4	7.0	5.4	7.1	7.4	7.2	6.0	6.0	7.3	6.9	6.1	7.4	7.2	6.7	5.0	5.9
24 hours	6.8	6.5	5.2	5.7	7.4	7.1	5.4	5.5	7.4	6.4	5.6	6.7	7.4	6.7	4.1	5.3

* N.S.—broth control without sugar.

TABLE V

Mean titratable acidity values of broth control and sugar broths inoculated with various organisms and incubated aerobically

TIME	L. ACIDOPHILUS				STREPTOCOCCI				YEASTS				YEAST AND L. ACIDOPHILUS			
	N.S.*	Sorb.	Dext.	Suc.	N.S.	Sorb.	Dext.	Suc.	N.S.	Sorb.	Dext.	Suc.	N.S.	Sorb.	Dext.	Suc.
Before inoculation	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
3 hours	4.5	4.4	4.8	4.4	4.0	4.0	4.4	3.1	3.9	3.3	3.9	3.7	3.7	3.5	3.6	3.7
6 hours	4.3	5.1	5.8	4.9	4.3	4.0	5.8	3.8	4.0	3.5	4.8	4.0	4.4	4.1	5.6	5.0
12 hours	5.6	6.2	7.2	5.3	4.3	4.2	7.0	5.0	3.8	3.6	8.6	3.9	5.0	6.6	6.8	5.9
24 hours	6.0	6.2	9.6	8.3	3.9	4.5	7.2	7.1	3.3	3.6	12.6	5.4	5.0	6.2	12.4	5.3

* N.S.—broth control without sugar.

the yeasts did not ferment so readily and the titratable acidity values were considerably greater. It will be noted that in several places the titratable acidity values appear inconsistent, i.e., there is not the progressive increase in the amount of NaOH used that would be expected. Possibly this variation might be explained in 2 ways, viz. due to the dark color of the medium it was difficult to detect small changes in the color of the indicator; and the small number of cultures used caused the mean values to be strongly influenced by extremes in the observed values of the individual cultures.

DISCUSSION

It is clear from the results of this study that under the conditions employed sorbitol is fermented much more slowly than either dextrose or sucrose when saliva or pure cultures are inoculated into media containing these sugars and incubated aerobically or anaerobically. As Fosdick, Campaigne and Fancher (7) have concluded, it is probable that it is the *rapid rate* of fermentation of carbohydrates on the enamel surface which is important in decalcification, for

TABLE VI

Mean pH determinations of broth control and sugar broths inoculated with various organisms and incubated anaerobically at 37°C.

TIME	L. ACIDOPHILUS				STREPTOCOCCI				YEASTS				YEAST AND L. ACIDOPHILUS			
	N.S.*	Sorb.	Dext.	Suc.	N.S.	Sorb.	Dext.	Suc.	N.S.	Sorb.	Dext.	Suc.	N.S.	Sorb.	Dext.	Suc.
Before inoculation	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
3 hours	7.4	7.2	6.7	7.2	7.4	7.3	6.7	7.2	7.5	7.4	6.7	7.4	7.3	7.2	6.7	7.1
6 hours	7.4	7.2	6.5	7.3	7.3	7.2	6.4	6.8	7.4	7.4	6.5	7.3	7.3	7.0	5.8	6.6
12 hours	7.4	6.8	5.0	7.0	7.4	7.0	5.0	6.2	7.5	7.5	6.6	7.3	7.5	7.1	5.3	6.2
24 hours	7.5	6.9	5.1	5.6	7.4	6.9	5.4	5.6	7.4	7.4	6.2	6.9	7.7	7.0	5.2	5.8

* N.S.—broth control without sugar.

TABLE VII

Mean titratable acidity values of broth control and sugar broths inoculated with various organisms and incubated anaerobically at 37°C.

TIME	L. ACIDOPHILUS				STREPTOCOCCI				YEASTS				YEAST AND L. ACIDOPHILUS			
	N.S.*	Sorb.	Dext.	Suc.	N.S.	Sorb.	Dext.	Suc.	N.S.	Sorb.	Dext.	Suc.	N.S.	Sorb.	Dext.	Suc.
Before inoculation	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
3 hours	5.8	3.5	8.3	3.2	8.3	8.0	11.4	8.9	4.2	6.3	8.3	5.5	4.2	5.1	6.4	5.0
6 hours	5.3	3.2	6.8	5.7	8.3	9.1	13.8	10.7	6.0	6.4	7.4	5.8	4.5	5.7	10.3	5.4
12 hours	5.9	8.2	21.2	10.2	8.3	10.0	16.0	13.0	6.0	6.3	9.6	6.7	4.9	5.7	11.6	3.7
24 hours	8.3	11.6	20.7	13.5	8.3	11.4	20.0	18.8	6.3	6.3	7.9	6.1	5.3	6.7	13.5	10.6

* N.S.—broth control without sugar.

substances which are only slowly fermented are washed off, buffered or diluted with saliva before the critical pH is reached. Fosdick found by determining the pH of carious material removed shortly after candy had been chewed that the pH drops sharply within a few minutes after the candy comes into contact with the carious material. In the present in vitro study it was found that no appreciable fermentation of dextrose or sucrose occurred until the cultures had been incubated 6–12 hours. It, therefore, seems obvious that other factors are involved in the in vivo fermentation in carious material than were indicated by

these in vitro studies. However, even though the rate of fermentation is accelerated in vivo, it is logical to assume on the basis of these studies that sorbitol will be fermented much more slowly than either dextrose or sucrose in carious material. It is planned to conduct studies on the rate of sorbitol fermentation in carious material, and should it be found that sorbitol is fermented just as rapidly as sucrose or dextrose, then it is evident that other factors are involved beside bacterial enzymes and we should modify our present-day concept of the stellar rôle of bacteria in the etiology of dental caries.

Since sorbitol was found to be fermented very slowly as compared with dextrose and sucrose, it is of interest to consider the cause of this delayed fermentation. It is generally believed, as stated by Kluyver (16), that bacteria oxidize sorbitol to sorbose by splitting off 2 hydrogen atoms on the second carbon atom to form water. The subsequent degradation products are not known, but it is assumed that a chain of reactions follows similar to that which is found in the fermentation of other carbohydrates (17). If these assumptions are true, it would seem that the initial oxidation of sorbitol to sorbose is the step accounting for the slow fermentation of sorbitol. To test this hypothesis, a small quantity of sorbose[6] was obtained and Trypticase broth containing M/10 concentrations was inoculated with saliva and incubated aerobically, the pH and titratable acidity values being determined in the usual manner. Due to the small quantity of sorbose available, only 4 samples of saliva were tested, but in each case the pH was reduced only from 7.2 to 7.0 after 24 hours incubation. It would thus appear that if sorbitol is oxidized to sorbose, bacterial enzymes also find it difficult to cause further decomposition of sorbose.

Since mannitol and dulcitol are sterioisomeres of sorbitol, it was thought of interest to determine the rate at which these sugar-alcohols would be attacked by oral microorganisms. Fifty-four samples of saliva were inoculated into Trypticase broth containing M/10 concentrations of mannitol and dulcitol[7] and pH and titratable acidity determinations made as previously described. In most samples the 2 sugars were slowly attacked and the pH and titratable acidity curves resembled those found for sorbitol. Apparently, microorganisms commonly found in the saliva have difficulty fermenting sugar-alcohols with the sterioconfiguration of the mannitol, dulcitol, sorbitol series. Mannitol and dulcitol do not possess the sweetening properties of sorbitol and are thus unsuited as substitute sugars.

SUMMARY

1. An attempt is described to make a quantitative estimation of the number of individuals whose saliva contains sorbitol-fermenting microorganisms, and the percentage of sorbitol-fermenters in the saliva. The technical difficulties involved in such a procedure probably invalidated the results obtained.

[6] The writer is indebted to Dr. N. K. Richtmeyer of the National Institute of Health for the sorbose used in this work.

[7] The mannitol and dulcitol used in this study were supplied through the courtesy of the Atlas Powder Co.

2. By inoculating 122 samples of saliva in nutrient broth or Trypticase broth containing M/10 concentrations of sorbitol, dextrose, and sucrose; the pH and titratable acidity developing in these cultures incubated for 24 hours at 37°C. under aerobic conditions were determined. Sorbitol was fermented much more slowly than either of the other sugars and seldom reached a pH of 5.0.

3. Fifty-five samples of saliva were incubated in a modified Brewer's thioglycollate medium for anaerobic cultivation and the developing pH values determined. Sorbitol, sucrose, and dextrose in M/10 concentrations were placed in this medium and the sorbitol was found to be fermented much more slowly than the other sugars.

4. Strains of *L. acidophilus*, oral streptococci, yeasts, and mixtures of yeasts and *L. acidophilus* were inoculated into Trypticase broth containing M/10 concentrations of sorbitol, dextrose, and sucrose. These test organisms were also inoculated into a modified thioglycollate medium containing M/10 concentrations of the above sugars. In all cases sorbitol was fermented more slowly than the other sugars.

5. The practicability of employing sorbitol as a substitute for sucrose or dextrose in the sweetening of foods, beverages, confections, etc. is discussed. On the basis of these in vitro studies, it is suggested that in view of the slow fermentation of sorbitol it might be useful in the prevention or control of dental caries. The cause of the delayed fermentation of sorbitol is discussed.

The writer wishes to acknowledge the technical assistance of Miss Matina S. Varipatis in this work.

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RADIOACTIVE PHOSPHATE ABSORPTION BY DENTIN AND ENAMEL[1]

GERRIT BEVELANDER AND MELVIN H. AMLER

The Departments of Anatomy, College of Dentistry and The Graduate School of Arts and Science, New York University, New York, N. Y.

INTRODUCTION

Studies have frequently been made on dentin in an attempt to clarify problems relating to the structure and physiological behavior of this tissue. Thus far, 3 principal methods have been used: 1. Dyes have been used by Beust (1), Fish (2, 3), Bodecker (4), Lefkowitz (5), and others in studies of the rate and degree of penetration through the dentin. 2. Roentgen ray absorption studies, to determine the relative absorption of different parts of the tooth, were made by Van Huysen, Hodge and Warren (6). 3. Radioactive isotopes have also been used extensively in studies relating to the absorption of dentin and other tissues.

Hevesy, Holst and Krogh (7) administered radioactive phosphorus to rats, cats and humans, and found that phosphorus atoms of the blood plasma exchange with those of the teeth and other tissues. They also state that actively growing teeth, such as the incisor of the rat, show a higher rate of absorption than mature teeth of the rat and other animals. They further state that the absorption of P_{32}, in the rat incisor, is much more pronounced in the rapidly-growing proximal part than in the distal region of the tooth. This situation was also found to be true in rat incisors in which the pulps had been removed. They conclude from these and other observations that the circumpulpal region of the tooth shows the highest degree of phosphorus absorption. Manly and Bale (8) measured the relative amount of radioactive pohosphorus absorbed by the glycocol extracted dentin of the rat incisor and observed results similar to those reported by Hevesy, Holst and Krogh. Observations regarding the differential absorption of radioactive phosphorus in the rat incisor have also been recorded by Erbacher and Wannenmacher (9).

The original observations of Hevesy, Holst and Krogh have also been confirmed in part by several other investigators. Wasserman, Blayney, Groezinger and DeWitt (10) observed in the dog that teeth with pulps removed take up radioactive phosphorus throughout the entire tooth at a rapid rate but to a lesser degree than in the intact tooth. Slight differences in the amount of absorption were noted by Volker and Sogannaes (11), and Sogannaes and Volker (12), who separated dentin into apical and coronal samples and also according to 'density' fractions. Finally, McCauley (13), and Scott and Marshall (14) noted in their radioautographs, that the circumpulpal dentin revealed a greater absorption of P_{32} than other parts of the tooth.

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PROBLEM

In this study, we have compared the relative P_{32} absorption of enamel and the different morphological varieties of dentin by 1: the in vitro study of human teeth, and 2 the in vitro and in vivo study of dogs' teeth. Studies previously reported, which deal with P_{32} absorption in teeth, have as a rule neglected to take into account the variation in the histological structure of dentin.

MATERIALS AND METHODS

Human teeth, obtained from routine extractions, were selected at random and sectioned mesio-distally on an abrasive wheel to a uniform thickness of from 200–300 μ. This permitted examination by microscope of the tissues to be measured. Dogs' teeth to be used for the in vitro tests were treated in a similar manner. The slabs were then immersed in a 10 cc. solution of P_{32}, having a concentration of approximately 20 μ/cc. To insure complete saturation, the slabs were boiled gently in this solution and then permitted to remain in it for 12 hours.

Following this procedure, the preparations were washed in tapwater and the cementum was then removed by means of an abrasive wheel. The teeth were dissected with the aid of a microscope so that the desired topographic and morphological varieties of dentin could be accurately identified and isolated. Each sample was rechecked under the microscope after preliminary separation. In large topographic areas, such as the circumpulpal zone, an abrasive wheel was used to trim away small amounts of excess tissue, and in the case of small areas, such as sclerosed tracts and abrasion cones, excess dentin was chipped away with a straight hand chisel.

20 to 25 teeth were used in each in vitro experiment and similar topographic or morphological varieties were pooled for subsequent treatment. This consisted of thorough pulverizing, desiccating and weighing the samples on an ordinary quantitative balance and then measuring their respective activities and expressing the results in microcuries per gram. The activites of the samples were measured with the use of a Geiger counter, and where the activites were too high, a simple electroscope with a calibrated galvanometer was used. The teeth from the in vivo experiments were handled in a manner similar to that just described.

Solutions of P_{32} in the form of Na_2HPO_4 were injected intraperitoneally into adult dogs on 1 or several successive days. The animals were sacrificed from 4 to 7 days after the first injection of radio phosphorus, and the teeth removed. These teeth were then sectioned, the appropriate regions dissected out under a microscope and the respective samples were desiccated, weighed and measured for their activities.

MORPHOLOGY

Before describing the results of our experiments, we wish to call attention to *fig. 1*, a semi-schematic drawing of a human adult tooth. In describing our experiments, we shall have occasion to refer to the different tissues or varieties

of dentin which are shown in this figure. Since the current terminology used in designating variations in dentin is not uniform, it is important to define our terminology precisely. This, we believe, can be most easily accomplished by the use of a carefully labeled drawing such as fig. 1.

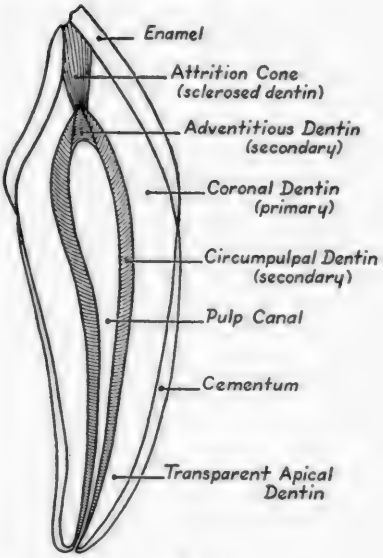

Fig. 1

OBSERVATIONS AND RESULTS

A. Human Teeth, in vitro tests:

The results of our experiments on human teeth which were designed to measure the relative amounts of P_{32} absorbed in different parts of the tooth are shown in Table 1. For purposes of convenience, all measurements have been recorded on a relative basis for each individual experiment. Furthermore, coronal dentin has been selected as a reference point and the value for this tissue designated as unity in each experiment.

As shown by the results tabulated in Table I, the absorption was tested for enamel and dentin. The dentin was separated on the basis of variation in topography, morphology, or both.

The 5 experiments reported here were performed on from 20 to 25 tooth slabs for each experiment recorded. The data in Table I show that the enamel was consistently lowest in the amount of P_{32} absorbed, measuring from 20% to 35% of the amount of phosphorus which was absorbed by coronal dentin. The variety showing the highest degree of absorption was always found to be the circumpulpal variety, which revealed a relative absorption of from 2 to 4 times as much as that absorbed by the coronal dentin. Transparent apical dentin absorbed amounts intermediate between the 2 varieties just mentioned having

relative values ranging between 1.1 to 1.37 times that observed in coronal dentin. The 3 varieties of dentin just referred to represent fairly definite topographic regions of the tooth. They also represent differences in structure of the tissue.

The relative degree of phosphorus absorption was also measured in several other varieties of dentin. These samples consisted of adventitious, in which absorption was about 1.5 times that observed in coronal dentin, and transparent sclerosed dentin, occurring under caries and attrition cones. The last 2 varieties exhibited absorptions considerably less than those of coronal dentin. It is

TABLE I

Relative absorption of radioactive phosphorus in different parts of teeth

Each set of measurements (I to V) represents data on pooled samples from 20 to 25 teeth

Tissue	I	II	III	IV	V
Enamel	—	.2	—	0.37	0.27
Dentin					
Coronal	1.0	1.0	1.0	1.0	1.0
Circumpulpal	3.3	4.2	2.1	2.7	2.1
Transparent Apical	—	1.2	1.1	1.3	1.37
Transparent Sclerosed	0.62	.9			
Adventitious		1.62			
Attrition Cone					0.82

TABLE II

Relative absorption of radioactive phosphorus in various parts of dogs' teeth

Each set of measurements (I to V) represents data on pooled samples from 10 teeth

	A. VITRO				B. VIVO			
Tissue	I	II	III	IV	I	II	III	IV
Enamel	0.5	0.45	—	—	0.27	0.5	—	0.35
Dentin								
Coronal	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Circumpulpal	2.0	3.3	1.5	1.96	2.7	1.6	4.3	2.8

apparent from the above presentation, that for each morphological variety of dentin tested, a different degree of P_{32} absorption was found.

B. Dog:

1. In vitro experiments:

The relative absorption of P_{32} was also studied in the teeth of the dog. The in vitro experiments were performed on pooled samples of tissues collected from the teeth of 4 dogs prepared and treated in a manner similar to that described in the previous experiment. In this study, the activity of the enamel and dentin was measured. The dentin was divided into 2 components differing morphologically and topographically: (1) coronal dentin, and (2) circumpulpal dentin.

The results of the in vitro studies on the dog are shown in Table IIA. Examination of the data incorporated in this table shows that enamel exhibited an activity (absorption) relatively lower than coronal dentin, and that circumpulpal dentin revealed absorption amounting to 2 to 3 times that of coronal dentin. These results are similar to those observed in the human teeth.

2. In vivo experiments:

The absorption of P_{32} by the enamel and 2 varieties of dentin in the living dog was studied according to the methods previously described. In performing these experiments, certain variables were introduced. Each dog differed with respect to the number of injections received, the length of time from the introduction of P_{32} until the animal was sacrificed, the total activity of the P_{32} injected, and the age and weight of the animal.

In Table IIB, are tabulated the absorption measurements for the 4 dogs subjected to in vivo experimentation. Each animal used constituted an individual experiment, and enamel, coronal, and circumpulpal dentin were measured and compared with the teeth of each of the other animals. In spite of the variables just referred to, the absorption of the same tissues in the different dogs was approximately the same. As shown in the table, the ratios obtained for these 3 samples, considering coronal dentin as unity, were .23 to .5 for enamel, and 1.6 to 4.3 for circumpulpal dentin.

DISCUSSION

In this study, we have been interested primarily in the relationship which exists between the absorption of radioactive phosphorus and the topographical and morphological variation in dentin. As previously stated, studies of this nature have been made with various dyes. At best, the use of dyes for this purpose gives a rough measurement of the degree to which they are absorbed by dentin.

The use of radioactive phosphorus in the measurement of absorption in a calcified tissue such as dentin has many advantages over the use of dyes for the simple reason that the amount of absorption can be of an extremely low order and yet be ascertained in a purely objective manner. Then, too, in in vivo experiments, the introduction of the phosphate ion is a less artificial process than the introduction of a dye or heavy metal.

Previous reports on the permeability of dentin to dyes have revealed that certain variations of this tissue are permeable or not permeable to a certain degree. Our experiments show that all topographical areas and all morphological varieties of dentin are permeable to P_{32} and absorb it in a measureable quantity. The degree to which these different types of dentin absorb this salt varies with the structure. When these results are compared with those of previous experiments of a similar nature, such as those of Hevesy, Holst and Krogh (7), and others, they are found to be in fairly close agreement insofar as these experiments correspond with ours. The postulation recently made by Bodecker (4) that in effect, absorption of radioactive phosphorus is dependent upon the structure of dentin has been shown to be true on the basis of our observations.

We do not believe, however, as Bodecker (4) implies, that absorption of dyes by dentin is more revealing or significant in either a morphological or physiological sense, than is the study of dentin by the absorption of the isotope P_{32}.

In comparing our results on human in vitro studies with those of Van Huysen, Hodge and Warren (6), who measured the density of dentin, the following correlation appears. We have recalculated their average density values and express coronal dentin as unity and other varieties on the same basis. If we arrange these values in decreasing order of density, they are with their respective density values as follows: attrition cone 1.1; coronal dentin 1; apical .94; adventitious .92. Our P_{32} absorption values for these varieties of dentin are as follows: attrition cone .82; coronal 1.0; apical 1.2; adventitious 1.6; and circumpulpal, which was not reported by Van Huysen, Hodge and Warren, 3.8. It is apparent, therefore, that P_{32} absorption decreases as density increases.

We wish, finally, to call attention to 2 other points which are of interest in connection with the absorption of P_{32}. The first of these is the repeated observations made by us that regardless of the condition of the experiment, that is, whether in vitro or in vivo, the circumpulpal zone invariably absorbs a much greater amount of P_{32} than any other part of the tooth. The second observation we wish to emphasize is that our measurements show that dentin absorbs P_{32} in both in vivo and in vitro conditions in the same relative order.

While we believe that these two observations are significant, we do not feel that our experiments are of such a nature as to allow us to try to place any specific interpretation upon them. Elucidation of these points must await further experimentation.

SUMMARY

1. A study of the relative amount of radioactive phosphorus which is absorbed by enamel and different topographic and morphological varieties of dentin has been ascertained.

2. Both in vitro and in vivo preparations show a direct relationship between variation in the morphology of dentin and the absorption of radioactive phosphorus. It has also been shown that the absorption of P_{32} is inversely related to the density of the dentin.

3. The relative amounts of P_{32} absorbed under conditions of in vivo and in vitro experimentation were observed to be similar for the enamel and the several topographical and morphological varieties of dentin which were studied.

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LOW CALCIUM DIET: DENTAL EFFECTS[1]

C. A. H. SMITH, D.M.D. AND R. F. LIGHT, B.S., M.S.

New York, N. Y.

Many discussions of the effect of various diets upon the teeth have emphasized the necessity for adequate amounts of calcium, phosphorus and vitamin D. It has been assumed that the withdrawal of any of these materials would seriously affect the teeth. However, this does not seem to be the case, for the withdrawal of calcium from the diet has never been shown to affect the tooth structure to any serious extent. Mellanby (1) concluded that "for the production of perfect teeth the diet must contain some calcium and phosphorus. If the diet is rich in vitamin D, the amount necessary to produce such teeth may be very small." Schour (2) stated, "A correlation between calcium metabolism and caries has not been established." Lund and Anderson (3), using white rats on a diet deficient in vitamin D and calcium, were unable to produce structural changes in the teeth of experimental animals, although the alveolar bone softened and the teeth loosened.

During the course of one of our studies material became available bearing out the conclusion that acute disturbances of the skeletal structure may occur with slight change in the dental structure.

PROCEDURE

Three dogs of terrier type, born March 9, 1942, were used in the study. The puppies were completely weaned at 1 month and on April 9th, dogs 106 and 109 were placed on a purified calcium-deficient diet consisting of 19% purified casein, 66% sucrose, 8% vegetable oil, 3% cod liver oil and 4% of a calcium and phosphorus free salt mixture. The animals were given daily supplements of purified vitamins of the B complex, plus a yeast extract. This diet contained 0.027% calcium and 0.153% phosphorus. Dog 110 was fed a similar diet except that it contained 45% crude casein with a corresponding reduction in the sucrose content. This diet had 0.196% calcium (derived from the casein) and 0.427% phosphorus. The animals received 100 gamma B_1, 100 gamma riboflavin, 100 gamma B_6, 1 milligram calcium pantothenate, 2 milligrams choline, 2 milligrams niacin, 20 milligrams inositol and 10 gamma biotin per kilo of body weight per day, plus 1.0 gram of yeast extract daily.

From May 12, 1942, animal 106 received supplemental calcium as $CaCO_3$ amounting to 120 mg of $CaCO_3$ per day which raised the calcium content of the diet, making it 0.10 to 0.15% of calcium, depending on the intake of food. From June 26, after 11 weeks of these diets, the animals received normal mineral

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supplements until they were sacrificed on October 1, 1942, after being on the experimental diets for 11 weeks and on the calcium and phosphorus supplemented

Fig. 1

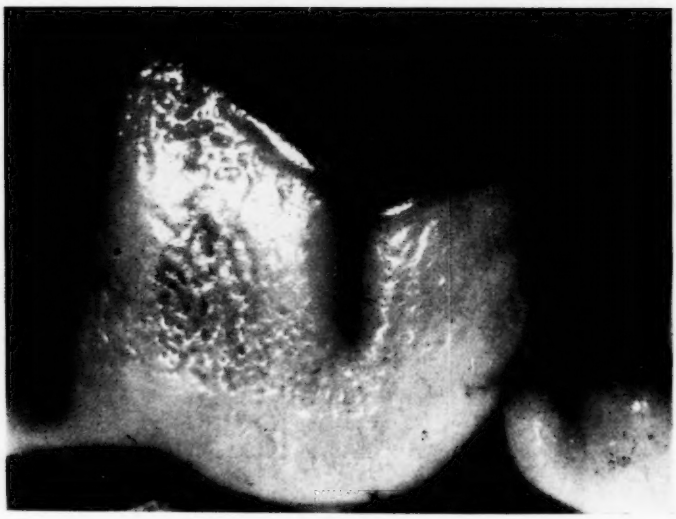

Fig. 2

Fig. 3

diet for 14 weeks. The supplemented diets contained 0.44% and 0.64% calcium respectively.

RESULTS

The animals grew reasonably well until the effects of the calcium deficiency became evident. The calcium deficiency produced a marked effect on skeletal development and is best illustrated by the radiographs of the forelegs of 109 (*fig. 1*). Although the end of the shaft of the ulna is somewhat enlarged, the uncalcified cartilagenous zone is narrow. The shaft of the ulna is bowed outwardly with the bend occurring in the central portion, indicative of an acute calcium deficiency. Further, animal 109 suffered a spontaneous fracture of the femur and a greenstick fracture of the left radius shortly after being changed to the normal diet. The fractures healed normally, although the animal was permanently deformed by the bent fore legs.

Mellanby (4) stated that the permanent teeth of dogs begin formation at 4 days of age and are completed in about 16 weeks, with eruption beginning at about 17 weeks. Thus, the 4 weeks before weaning, plus the 11 weeks on diet (15 weeks), placed the full effect of the low calcium diet upon the permanent teeth during the entire period of their development.

Gross examination of the jaws shows a slight hypoplasia of the enamel in each animal (*fig. 2*). The roughening did not in any place expose the dentin. The teeth were tightly fixed in their alveoli and the radiographs show normal alveolar structure (*fig. 3*).

Ground sections showed the hypoplasia of the enamel and dentin but, together with decalcified and stained sections, show no further modification of tooth structure.

Bowes and Murray (5) have shown that in hypoplasia the total ash of the enamel is decreased from 95.38% to 94.67%, while the calcium decreased from 37.07% to 35.81%. They found less variation in the dentin. While hypoplasia may be produced by various means, it would seem that low calcium content of the enamel is characteristic of the condition.

CONCLUSIONS

These observations indicate that an acute calcium deficiency in the diet of young dogs may produce drastic effects on the skeletal structure, yet have little effect on the structure of the teeth and alveolar process. A deficiency of calcium that produced spontaneous fractures, bending of the long bones and failure of growth during the period in which the permanent teeth were formed, did not result in severe hypoplasia of the enamel nor in other structural modifications.

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